Approach to the Synthesis of Aza-Analogues of Narciclasine through an Intramolecular Heck Reaction

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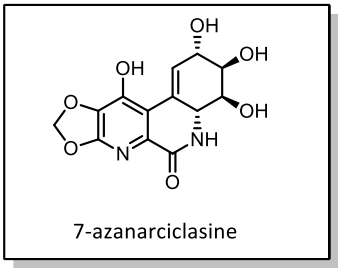
ABSTRACT

This thesis describes work towards the total synthesis of a 7-aza analogue of the *Amaryllidaceae* alkaloid narciclasine, a potent anticancer compound which suffers from a poor solubility profile. A key strategy in the formation of the C-ring is the biotransformation of bromobenzene by *E.coli* JM109. The densely substituted heterocyclic A-ring is obtained by sequential directed *ortho*-metalation and the fragment union accomplished with an amide coupling and subsequent intramolecular Heck reaction.



7-azanarciclasine

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LIST OF ABBREVIATIONS

2,2-DMP	2,2-dimethoxypropane
AcOH	Acetic acid
AIBN	2,2'-Azobis(2-methylpropionitrile)
Boc	Di-*tert*-butyl dicarbonate
Bz	Benzoyl
CBz	Carboxybenzyl
DBU	1,8-Diazabicycloundec-7-ene
DIPEA	*N,N*-Di*iso*propylethylamine
DIBAL-H	Di*iso*butylaluminium hydride
DMAP	4-Dimethylaminopyridine
DMP	Dess-Martin periodinane
DPPA	Diphenylphosphoryl azide
DPPE	1,2-*Bis*(diphenylphospino)ethane
DIPHOS	Ethylenebis (diphenylphosphine)
ED_{50}	Effective dose 50%
EE	Ethoxy ethyl
GI_{50}	Growth inhibition 50%
HBTU	*O*-Benzotriazole-*N,N,N',N'*-tetramethyluronium hexafluorophosphate
IC_{50}	Half maximal inhibitory concentration

IPA	Isopropyl alcohol
LTMP	Lithium tetramethylpiperidide
m-CPBA	*m*-Chloroperoxybenzoic acid
p-MBDMA	p-Methoxybenzaldehyde dimethylacetal
MOM-Cl	Chloromethyl methyl ether
NEt_3	Triethylamine
NBS	*N*-Bromosuccinimide
n-BuLi	*n*-Butyllithium
NH_4OH	Ammonium hydroxide
NMO	*N*-Methylmorpholine-*N*-Oxide
PMP	*p*-methoxybenzyl
PPTS	Pyridinium *p*-toluenesulfonate
UHP	Urea hydrogen peroxide
t-BuLi	*t*-Butyllithium
TBSCl	*t*-Butyldimethylsilyl chloride
TEBAC	Benzyltriethylammonium chloride
TFA	Trifluoroacetic acid
TMEDA	Tetramethylethylenediamine
TMNO	Trimethylamine *N*-oxide
Troc	2,2,2-Trichloroethyl chloroformate

1. Introduction

The plants from the *Amaryllidaceae* family have long been under scrutiny for the medicinal properties of their extracts. The present scientific evidence shows that the isocarbostyril constituents of the *Amaryllidaceae* family, particularly narciclasine **1** and pancratistatin **2**, and their congeners are the metabolites that are most responsible for the therapeutic benefits in the treatment of cancer. However, these compounds have issues inhibiting their development into drug candidates, such as poor solubility in water, densely functionalised structure, low natural abundance, and an unknown mechanism of action.

Narciclasine **1** was isolated and discovered by Ceriotti[1] in 1968 and pancratistatin **2** by Pettit[2] in 1984 (Figure 1), these two compounds were found to have antineoplastic activity when they were screened for possible drugs. Unfortunately, although these compounds were potent, solubility is a problem. Chemists attempted to discover possible analogues that will not only solve the issue of solubility but also further increase the biological activity.

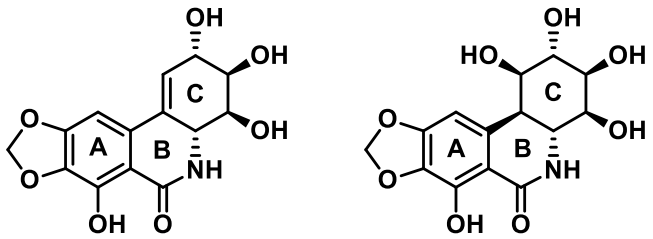


Figure 1- Structure of Narciclasine and Pancratistatin

The main objective of this project is to construct the A-ring aza-analogues of narciclasine that have the same or greater biological activity and to solve the solubility issues that have been found with this family (Figure 2). A key principle of the Hudlický group approach to synthesis is that the process should be efficient, elegant, and environmentally benign. The rapid construction of the desired functionality and the assembly of the fragments to yield the target compound exemplify these principles, with the synthesis having a convergent approach that begins with a 'green' starting material. The goal of this research project is to produce various structural analogs for the investigation of structure-activity relationship (SAR) studies in order to determine whether analogues possess comparable or increased anticancer activity. In order to be a drug candidate it has to exhibit improved water solubility, and our approach to this problem is the introduction of a nitrogen atom, which we propose will enhance the polarity and hence hydrophilicity of the compounds.

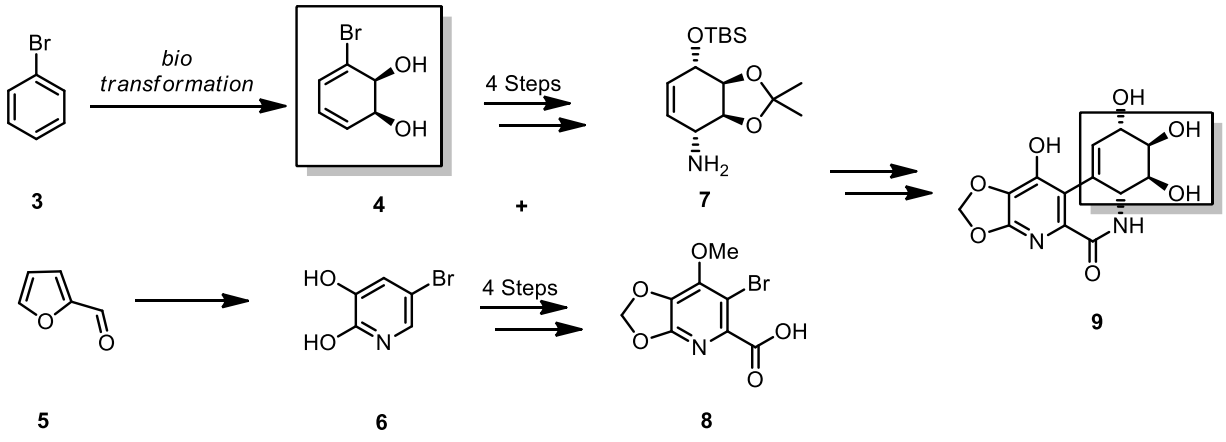


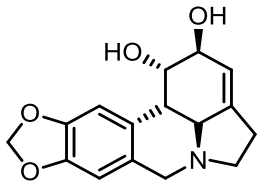
Figure 2- General Strategy for the synthesis of A-ring hetero-analogues of Narciclasine

2. Historical

2.1. *Amaryllidaceae* Alkaloids

2.1.1. Discovery and Biosynthesis

The anticancer properties of *Amaryllidaceae* extracts were discovered by Hippocrates of Kos, also known as Father of Medicine, from the oil of *Narcissus poeticus L.* The oil was used for the treatment of uterine cancer. His successors, Pedanius Dioscorides and Soranus of Ephesus, continued using these treatments for many years.[3] There are specific metabolites that are responsible for these anticancer properties. The first modern in-depth study began with the isolation of lycorine **10** from *N. pseudonarcissus* in 1877 (Figure 3).[4] The main focus of this section will be on components possessing the isocarbostyril structural motif **11**.



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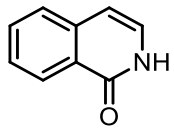

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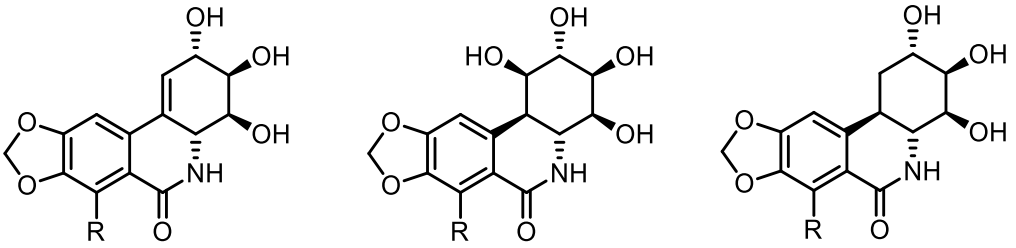
Figure 3- The Structure of Lycorine and isocarbostyril moiety

In the 20th century, the alkaloids that possessed the highest anticancer activity were isolated and found to contain an isocarbostyril moeity. Narciclasine **1** was discovered in 1968,[1] which was closely followed by 7-deoxynarciclasine (lycoricidine,

12).[5] Twenty years later, Pettit[2] discovered pancratitastin **2** in 1984, and Ghosal[6] isolated 7-deoxypancratistatin **13.** In 1975, a semi-synthetic route to *trans*-dihydronarciclasine **14** was published,[7] but was isolated from natural source in 1990 by Pettit.[8] The last isolation of the narciclasine species was 7-deoxy-*trans*-dihydronarciclasine **15** in 1993 by Pettit.[9]



1 R=OH
12 R=H

2 R=OH
13 R=H

14 R=OH
15 R=H

Figure 4- Three major isocarbotyril congeners and 7-deoxy versions

The biosynthesis of the *Amaryllidaceae* alkaloids has been investigated and described extensively in Kornienko's 2008 review.[3] The pivotal intermediate in the biosynthesis, *O*-methylnorbelladine (**16,** Figure 5) is derived from amino acids phenylalanine **17** and tyrosine **18**. Through a series of biotransformations, the derived amino acids procatechuic aldehyde **19** and tyramine **20** condense to afford Shiff base **21**. The reduction of **21** yields norbelladine **22** and in turn is methylated to *O*-methylnorbelladine **16**. There are two types of coupling pathways in this class of alkaloids, *para-ortho* oxidative or *para-para*. Radioactive labeling studies concluded that narciclasine **1** follows a *para-para* phenol oxidative pathway.[10] Oxidation of the *O*-

methylnorbelladine and subsequent aza- Michael addition the noroxomaritidine **23** is formed, after a series of oxidation steps of the alkaloid narciclasine **1** is produced.

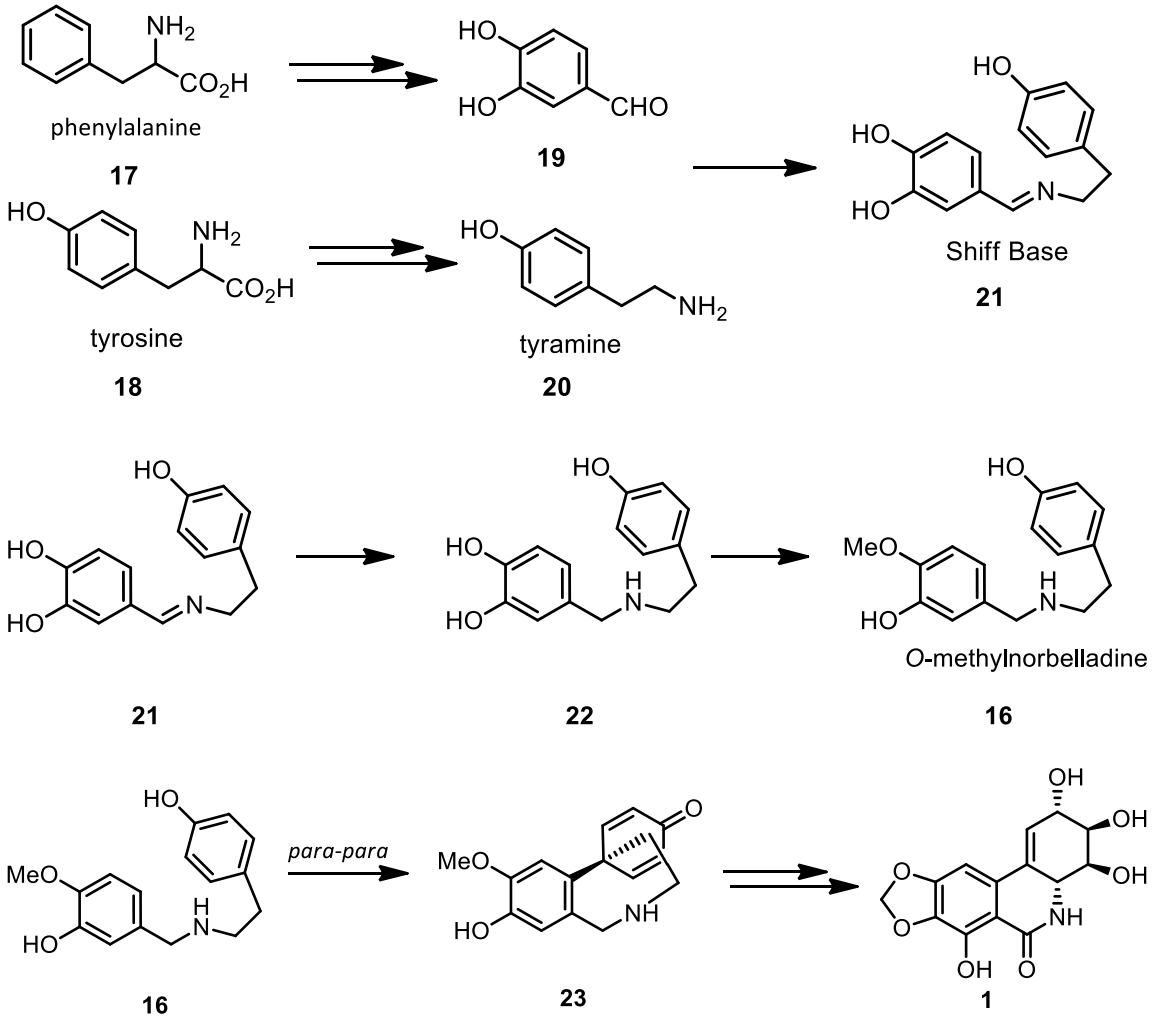
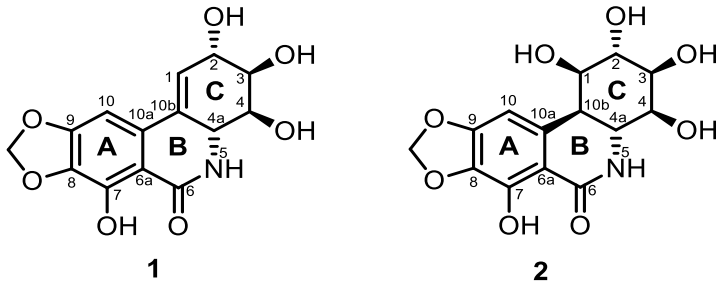


Figure 5- The Biosynthetic Route to Narciclasine

2.1.2. Selected Total Syntheses of Narciclasine

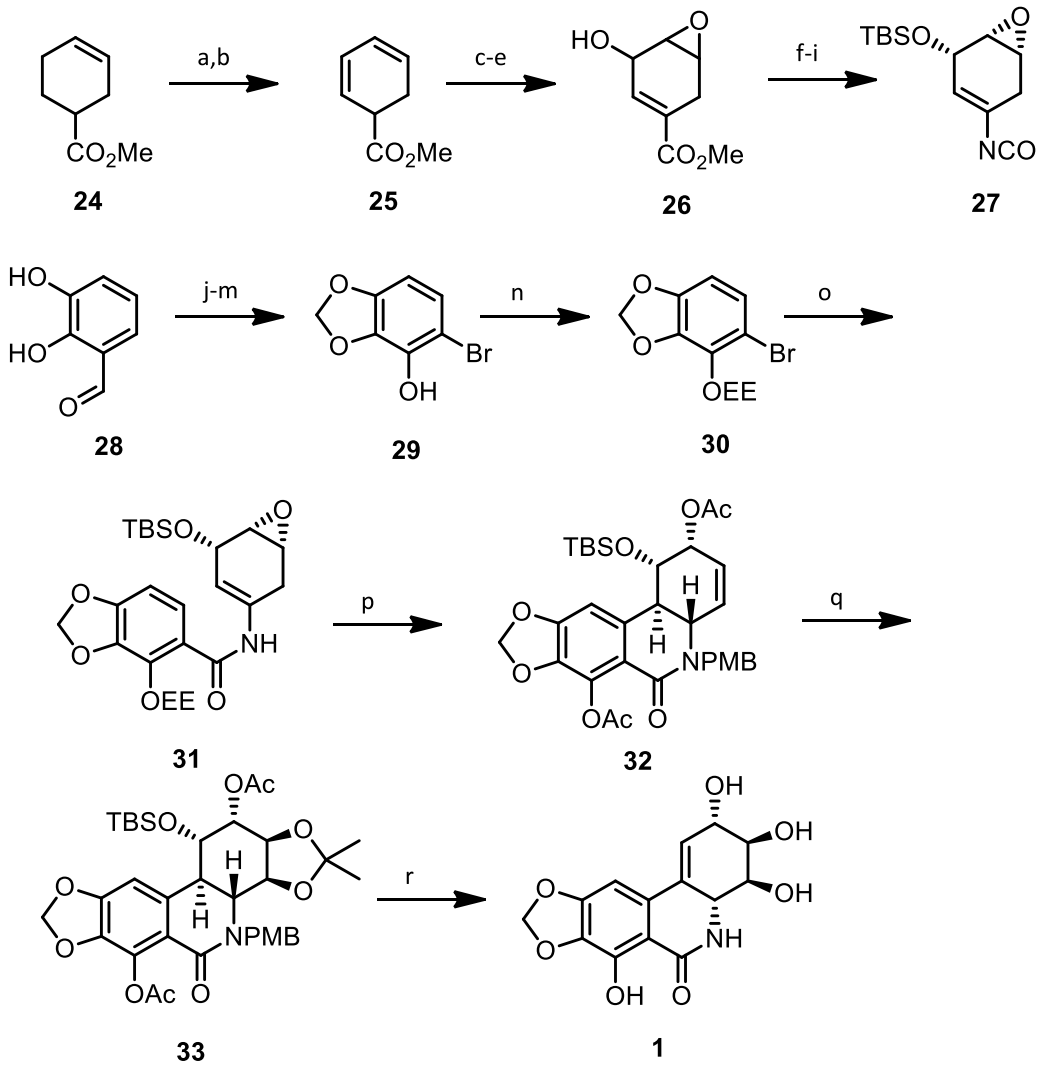
Although narciclasine **1** was isolated in 1968, it was not successfully synthesized until 1997 by Rigby. This section will not cover all total syntheses of narciclasine , but a select few published by Rigby,[11] Keck,[12] Hudlicky,[13] and Yan.[14] In order to follow the nomenclature and numbering in this document, the system is displayed in Figure 6.



1 **2**

Figure 6- Narciclasine and Pancratistatin nomenclature and numbering system

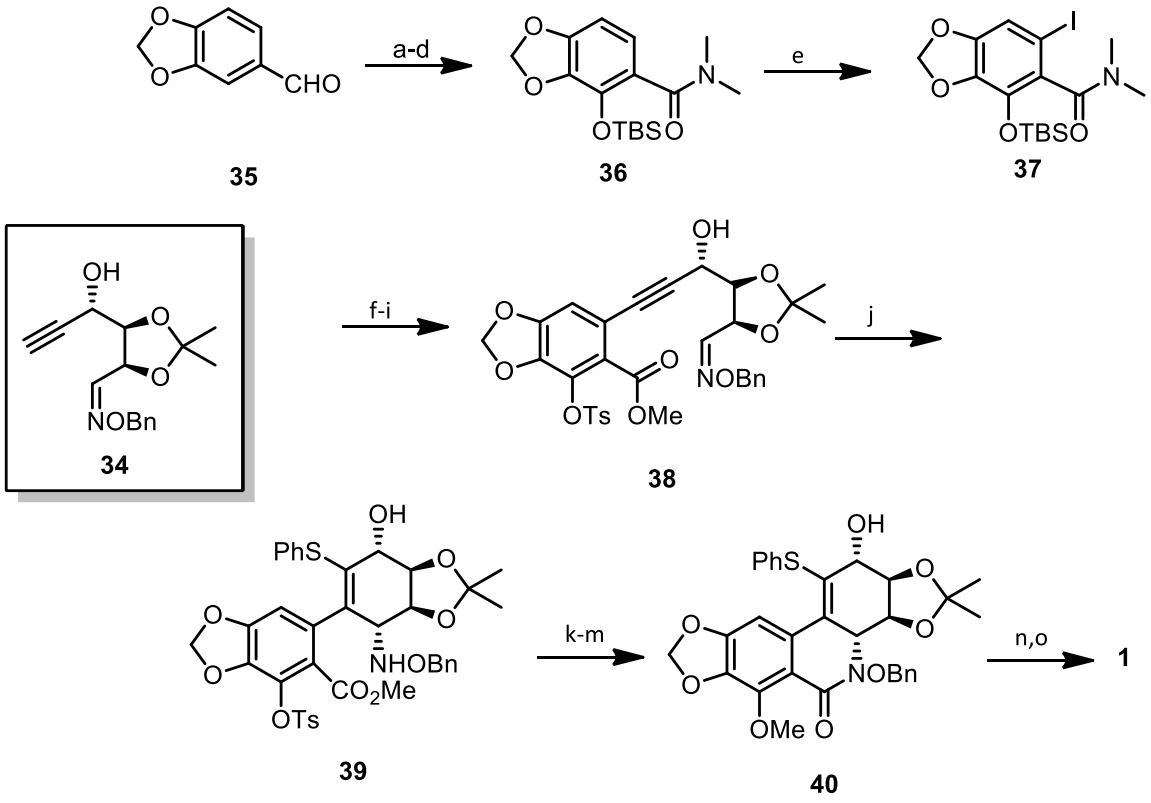
The first enantioselective synthesis of narciclasine was done by Rigby[11] in 1997 (Scheme 1). The preparation of the *syn*-epoxy alcohol **27** began by transforming the methyl ester **24** by Berchtold conditons.[15] The A-ring fragment **29** was prepared in four steps from 2,3-dihydroxybenzaldehyde **28**. Before the cyclization of A and the C-ring, the hydroxyl is protected by ethoxy ethyl ether **30**. In order to couple the two fragments together, the carboxylic acid **27** is converted to an isocyanate and methylated which led to the formation of amide **31**. After the installation of *p*-methoxybenyzl group, a photochemical enamide cyclization was done. With the use of diphenyl diselenide, the epoxide is opened followed by the acylation and protection of the diol by acetonide **32**. The removal of all protecting groups results in the first enantioselective total synthesis of narciclasine **1** in 27 steps.[11]



a) NBS, AIBN, PhH, reflux; b) AIBN, Bu_3SnH, PhH, 75%; c) $^1O_2$, hv, rose bengal; d) $(Ph_3P)_2RuCl_2$, CH_2Cl_2; e) NaOMe, MeOH, 30%; f) *n* PrCOCl, NEt_3; g) cholesterol esterase; h) TBSCl, imidazole; i) i)LiOH, MeOH, H_2O, 42%; ii) DPPA, NEt_3, PhH; j) CH_2Br_2, K_2CO_3; k) *m*-CPBA; l) KOH/EtOH; m) CF_3CO_2Ag, Br_2, 45%; n) ethyl vinyl ether, PPTS, 70%; o) i) *n*-BuLi, THF, -78 °C, **27**, 52%; p) i) PMBBr, NaH; ii) PPTS, MeOH, 76%; iii) hv, PhH, 46%; iv) $(PhSe)_2$, $NaBH_4$; v) NaH, AcCl, 48%; q) i) OsO_4, TMNO, t-BuOH; ii) TsOH, $(CH_3)_2C(OMe)_2$, 76%; r) i)TBAF, THF; ii) Burgess Reagent, 64%; iii) K_2CO_3, MeOH; iv) *n*-BuLi, THF, O_2; v) TsOH, 37%.

Scheme 1- Rigby's Enantioselective Total Synthesis of Narciclasine

Keck and colleagues[12] did an in-depth study of which protecting group is ideal for the 7-hydroxyl of **1** (Scheme 2). The aldehyde **35** was converted to **36** according to the procedure published by Gilman,[16] followed by metalation using *n*-BuLi with trimethyl borate and hydrogen peroxide is added to form the hydroxyl which is subsequently protected by TBS-Cl. After the second metalation and quenching by iodine, aryl iodide **37** was obtained. During the conversion of the dimethyl amide to methyl ester TBS was also removed, therefore the protecting group was altered to tosylate which can possibly be removed during the SmI_2 reduction step. Songashira coupling of **34** with **37** afforded **38**, soon after the radical cyclization. The cyclization, reductive removal of thiophenol and tosylate proceeded in one step, however the reaction was slow and low yielding. Alternatively a stepwise approach which included deprotection, cyclization, and removal of thiophenol to afford **1** with the overall yield of 26% in 14 steps.

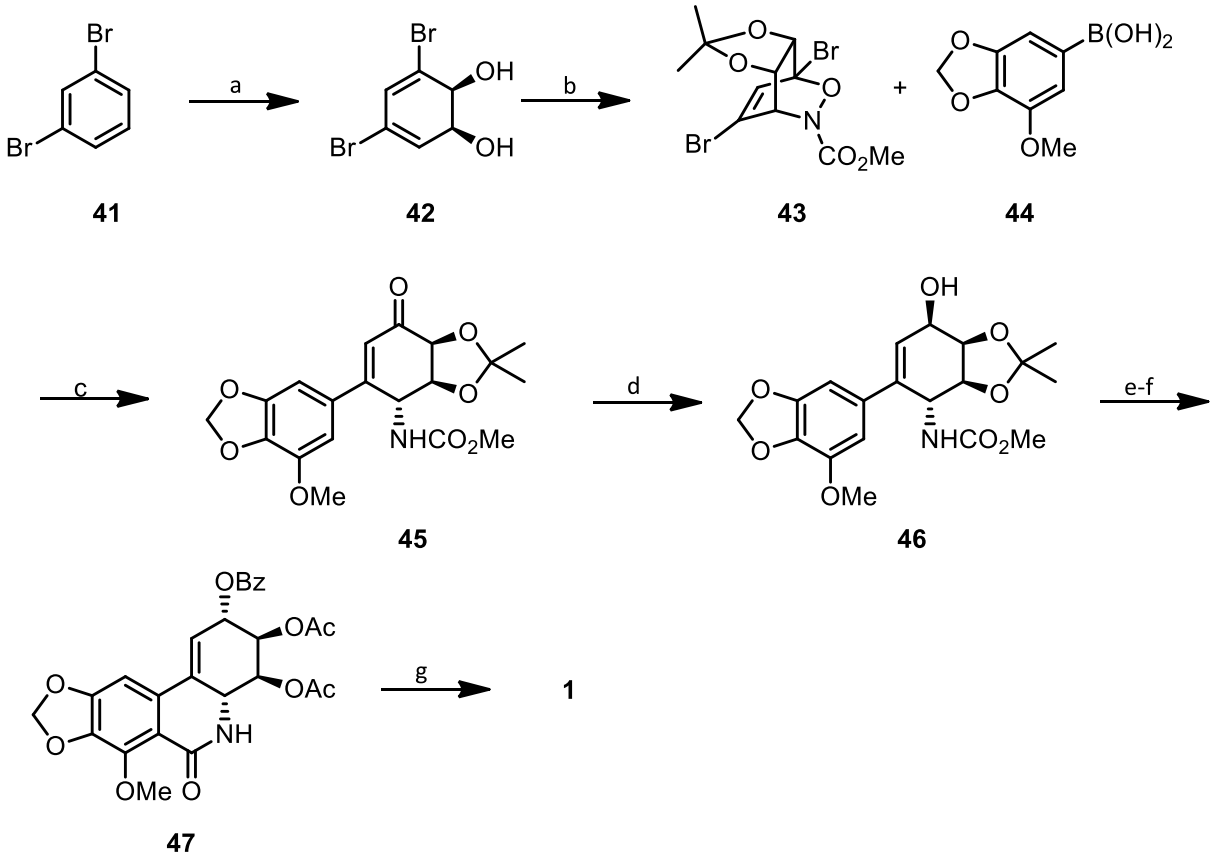


a) MnO_2, NaCN, Me_2NH, IPA, 95%; b) *n*-BuLi, TMEDA, -104 °C, $B(OMe)_3$, THF c) HOAc, H_2O_2, 78%; d)TBSCl, imidazole, 95%; e) *n*-BuLi, TMEDA, -104 °C, Et_2O, I_2, 72%; f) Me_3OBF_4, Na_2HPO_4, MeCN, 91%; g) MeI, K_2CO_3, 82%; h)TsCl, pyridine, 86%; i) $Pd(OAc)_2$, CuI, NEt_3, **34**, PPh_3, THF, 75%; j) PhSH, hv, 27 °C, 73%; k) SmI_2,THF, H_2O, 0 °C, 94%; l) MeI, K_2CO_3, DMF, 96%; m) Me_3Al, THF, -15-65 °C; 72%; n) SmI_2, MeOH, THF, 0 °C, 2h, 87%; o) TFA, 0 °C, 89%

Scheme 2- Keck's Total Synthesis of Narciclasine

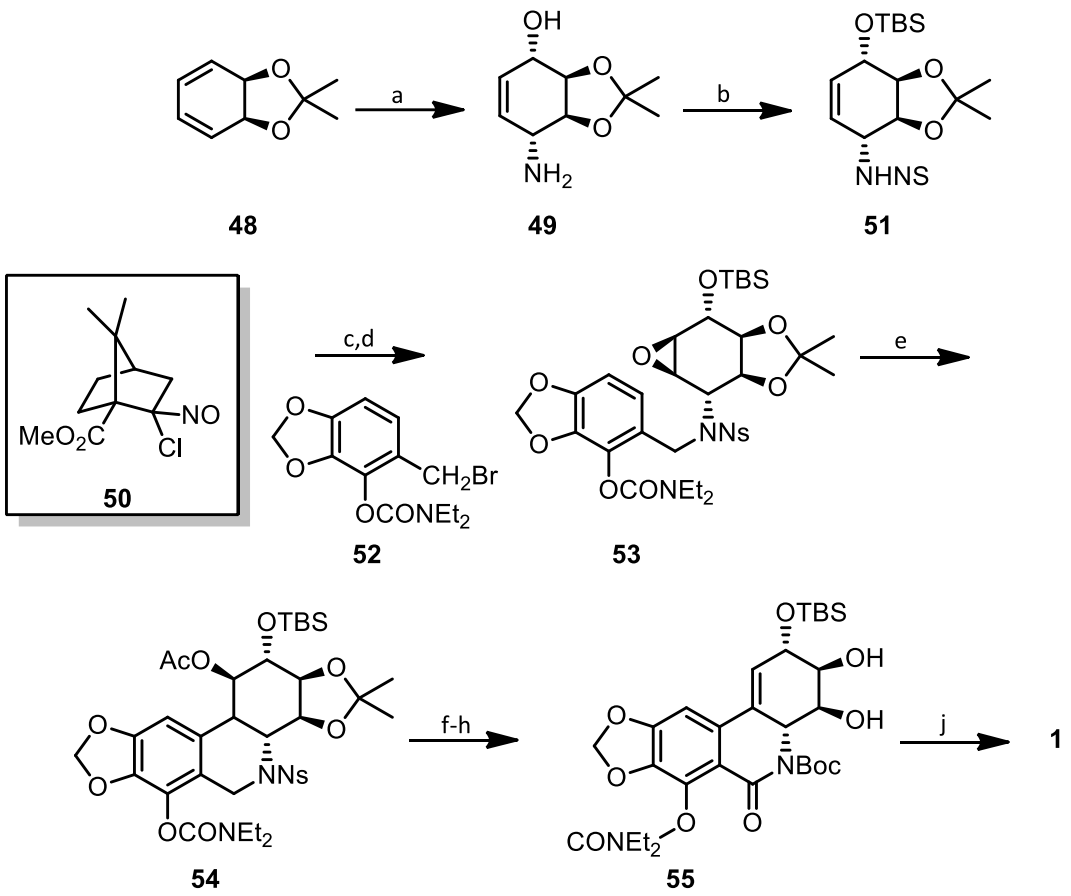
The chemoenzymatic dihydroxylation has been a long standing procedure in the Hudlicky's lab (Scheme 3).[13] This useful transformation was firstly described by Gibson.[17] The m-bromobenzene **41** is subjected to whole-cell fermentation with *E.coli* JM109 (pDTG601A) yielding diol **42**. Subsequently **42** was converted to the bicyclic oxazine **43**, which then reacts with **44** via Suzuki coupling to produce **45**. The reduction of enone using $NaBH_4$ delivered the undesired stereochemistry on carbon 2, therefore under

Mitsunobu conditions the correct stereochemistry was obtained to yield **47**. The closure of ring B was done by a modification of the Bischler-Napieralski,[18] all protecting groups were removed and **1** was obtained in 12 steps.



a) *E.coli* JM109 (pDTG601A), 4g/L; b) DMP, acetone, TsOH, rt, $NHCO_2Me$, $NaIO_4$, rt, 70%, Al(Hg), THF, 80%; c) i) **44**,$Mo(CO)_6$, MeCN-H_2O, reflux, 75%; ii) $Pd(PPh_3)$, aq. Na_2CO_3, PhH, reflux, 30%; d) $NaBH_4$, $CeCl_3$, MeOH, 0 °C , 80%; e) BzOH, Bu_3P, DEAD, THF, rt, 65%; f) i) Dowex 50X8-100, MeOH, rt; ii) Ac_2O, pyridine, DMAP, rt, 70%; g) i) Tf_2O, DMAP, CH_2Cl_2, 0 °C, 40%; ii) Amberlyst A21, MeOH, rt; iii) LiCl, DMF, 120 °C, 20%.

Scheme 3- Hudlicky's Total Synthesis of Narciclasine



a) i) **50**, CH_2Cl_2; ii) Al(Hg), MeCN, 85%; b) i) NsCl, DBU, NEt_3, MeCN; ii) TBSCl, DBU, rt, 78%; c) NBS, acetone, H_2O, 98%; d) **52**, K_2CO_3, MeCN, 88%; e) i) $SnCl_4$, CH_2Cl_2; ii) Ac_2O, K_2CO_3, 98%; f) thioglicolic acid, LiOH, DMF, 78%; g) i) Boc_2O, MeCN; ii) $RuCl_3$, $NaIO_4$, H_2O, 67%; h) DBU, benzene, 96%; j) i) HCOOH, THF; ii) LAH, THF, 65%.

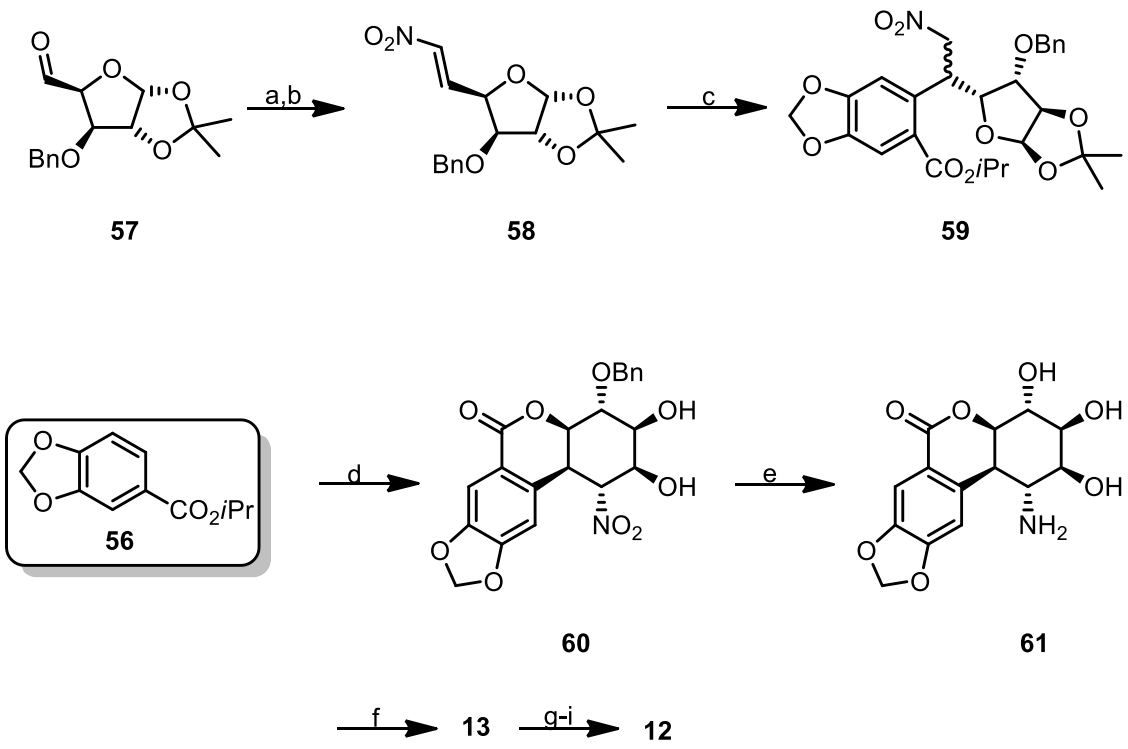
Scheme 4- Yan's Total Synthesis of Narciclasine

The most recent synthesis of **1** was reported by Elango and Yan in 2002 (Scheme 4).[14] In order to prepare the conduramine **49**, a one-pot cycloaddition of **48** and **50** was carried out and a reduction with Al(Hg). Protection of **49** gave conduramine **51** which was further reacted with NBS to produce bromohydrins, subsequently reacting with **52** to yield the epoxide **53**. Formation of ring B is mediated by Lewis acid catalyzed intramolecular arene coupling to yield **54.** After the removal of nosyl, the compound

was treated with Boc_2O, following the addition of $RuCl_3$ and $NaIO_4$ in order for the benzylic oxidation to occur. After heating the reaction mixture with DBU to obtain the 10a-10b bond after a series of deprotection steps, **1** is obtained in 9 steps with an overall yield of 19%.

2.1.3. Total Syntheses of Lycoricidine

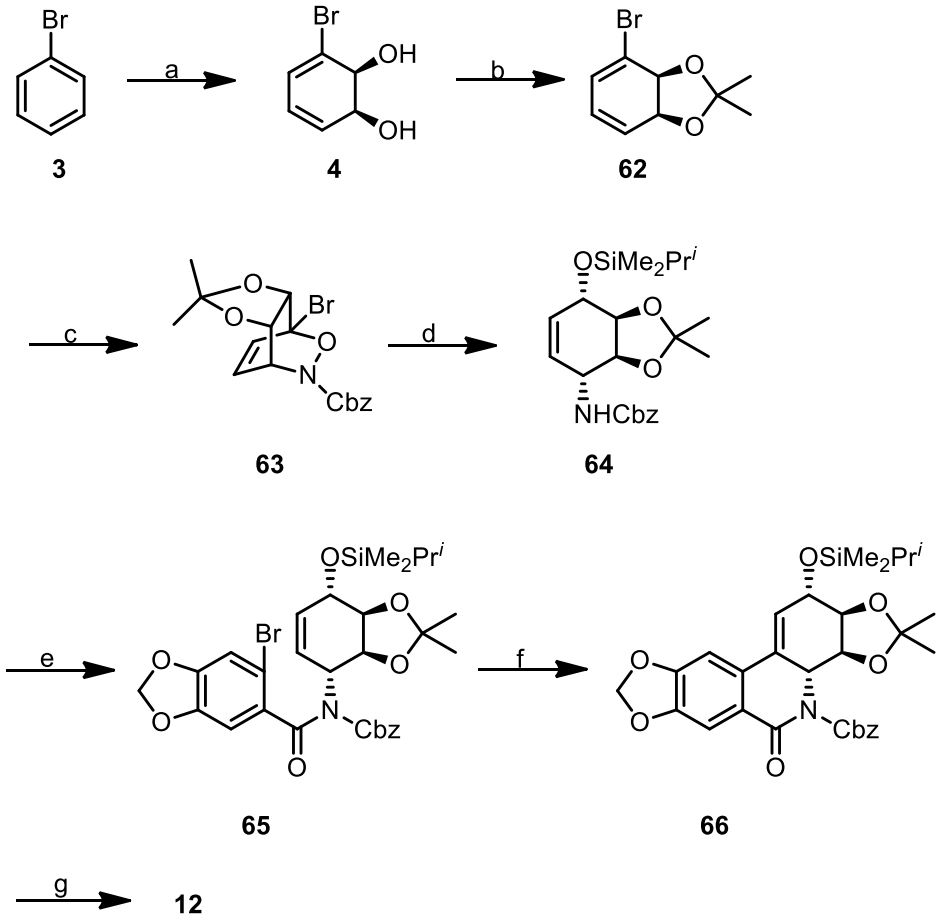
Lycoricidine **12** was isolated from the bulbs of *Lycoris radiata* in 1968 by Okamoto.[5] In 1975 the first racemic total synthesis was published by Ohta,[19] but it was not until 1982 when the first enantioselective synthesis of **12** was achieved by Paulsen and Stubbe (Scheme 5).[20] Since this first publication, many selective and efficient syntheses have been developed by Ogawa,[21] Hudlicky,[22] Keck,[12] and Yan.[23]



a) i) CH_3NO_2, NaOH; ii) Ac_2O, TsOH, 75%; b) K_2CO_3, benzene, 71%; c) **56**, *n*-BuLi, THF; d) i) AcOH, H_2O; ii) $NaHCO_3$, MeOH, 34%; e) H_2, Pd/C, MeOH, 77%; f) K_2CO_3, MeOH, 72%; g) BzCl, pyridine, DMAP; h) $SOCl_2$, pyridine; 70% for 2 steps; i) NH_3, MeOH.

Scheme 5- First Enantioselective Synthesis of Lycoricidine

Paulsen and Stubbe successfully achieved an asymmetric synthesis by starting from glucose derivative **57**, Scheme 5.[20] The aldehyde **57** was transformed to the nitroalkene **58** using a Henry reaction, which was reacted with lithiated **56** to produce **59.** The deprotection of the acetonide afforded the cyclized product **60**, after which reduction and recyclization of **61** led to another one of the *Amaryllidaceae* alkaloids, 7-deoxypancratistatin **13**. By selective reprotection and elimination the desired product lycoricidine **12** was synthesized.



a) *Pseudomonas putida*; b) 2,2-DMP, acetone, *p*-TsOH; c) benzyl hydroxycarbamate, Bu_4NIO_4, CH_2Cl_2; 74% d) i) Al(Hg), THF, 91%; ii) $ClSiMe_2Pr^i$, Imidazole, CH_2C1_2, 98%; e) i) *n*-BuLi, THF, -78 °C; ii) Br-piperonyloyl chloride, 77%; iii)$Pd(OAc)_2$, TI(OAc), DIPHOS, anisole, 27%; f) Pd(C), cyclohexene, EtOH, 99%; g) i) CF_3CO_2H, 0 °C, 85%.

Scheme 6- Hudlicky's Total Synthesis of Lycoricidine

Before the report of Hudlický's 1992 (+)-Lycoricidine synthesis, there was none shorter than 15 steps (Scheme 6).[22] The bromodiol **4** obtained from bromobenzene *via* bacterial dioxygenase of *Pseudomonas putida,* was protected with 2,2-DMP to yield **62**. The Cbz-protected oxazine **63** underwent reduction cleavage of N-O to afford **64** using aluminum amalgam. The closure of **65** were first done using Chida's conditions,[21] unfortunately it cannot be reproduced, therefore a modified Heck cyclization with

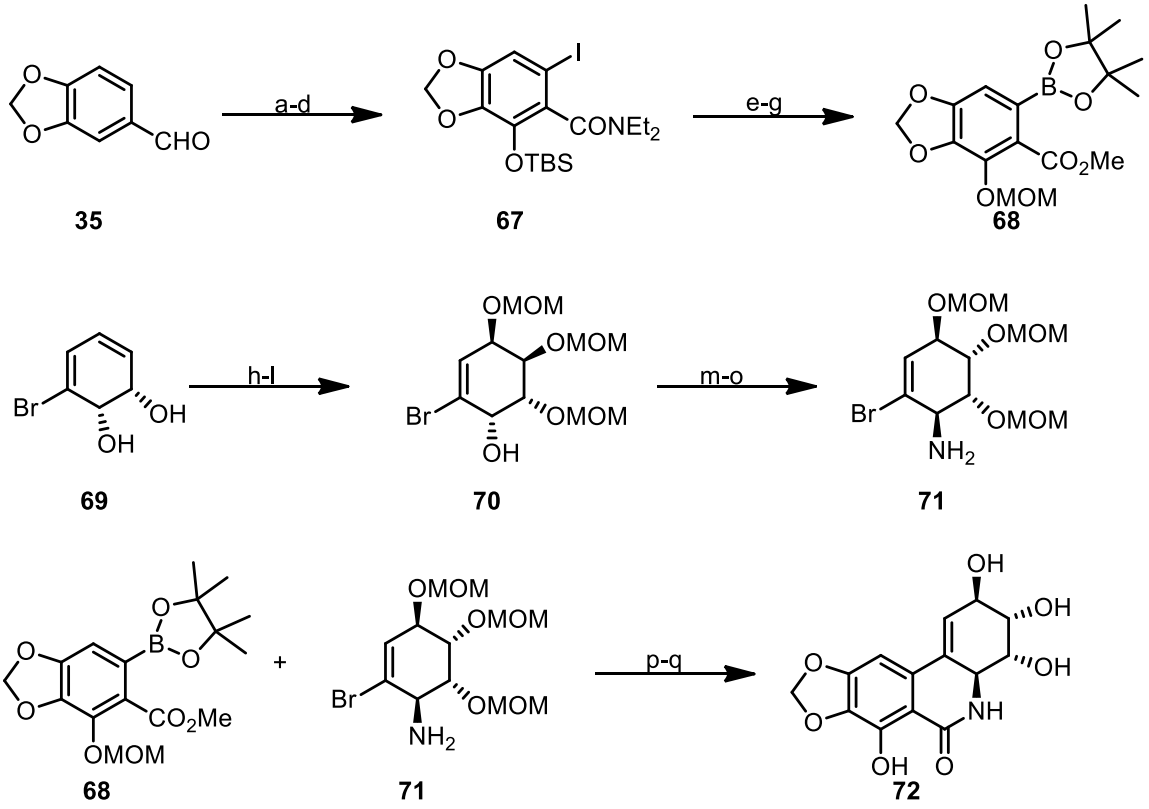
$Pd(OAc)_2$, Tl(OAc), and DIPHOS was used to yield the desired product **66**. The total synthesis of lycoricidine was achieved in 9 steps.

2.1.4. Synthesis of Analogues of Narciclasine and Lycorcidine

Although these *Amaryllidaceae* alkaloids are potent, the problem of insolubility has halted the possibility of using this class of compounds as a therapeutic. There have been extensive studies towards finding possible analogues, not only with the same potency, but solving the problem of solubility. This section will briefly cover total syntheses of different narciclasine and lycorcidine analogues from Banwell,[24,25] Chapleur,[26,27] and Kim.[28]

Banwell published the total synthesis of *ent*-narciclasine in 2008.[24] To assemble the aromatic core the majority of the procedures were taken from Keck's total synthesis of narciclasine.[12] The aldehyde **35** (Scheme 7) is carefully functionalized by first transforming the aldehyde to an ethyl amine, directed *ortho*-methalation to add the hydroxyl and the iodide resulting compound **67**. The conversion of the ethyl amine to the ester resulted in the cleavage of the TBS group and therefore MOM-Cl was used to protect the hydroxyl before the compound was subjected to the Miyaura borylation to afford **68**. The assembly of the C-ring core begins with *cis*-bromodiol **69**. In order to obtain **70**, the diol is protected as a *cis*-1,2-dihydroxylatio, done using UpJohn conditions, which proceeds with excellent regio- and diastereo-control. The alcohol groups were protected with MOM, reductive cleavage is performed by DIBAL-H to remove PMP. To selectively deprotect the desired hydroxyl DDQ was used and in turn

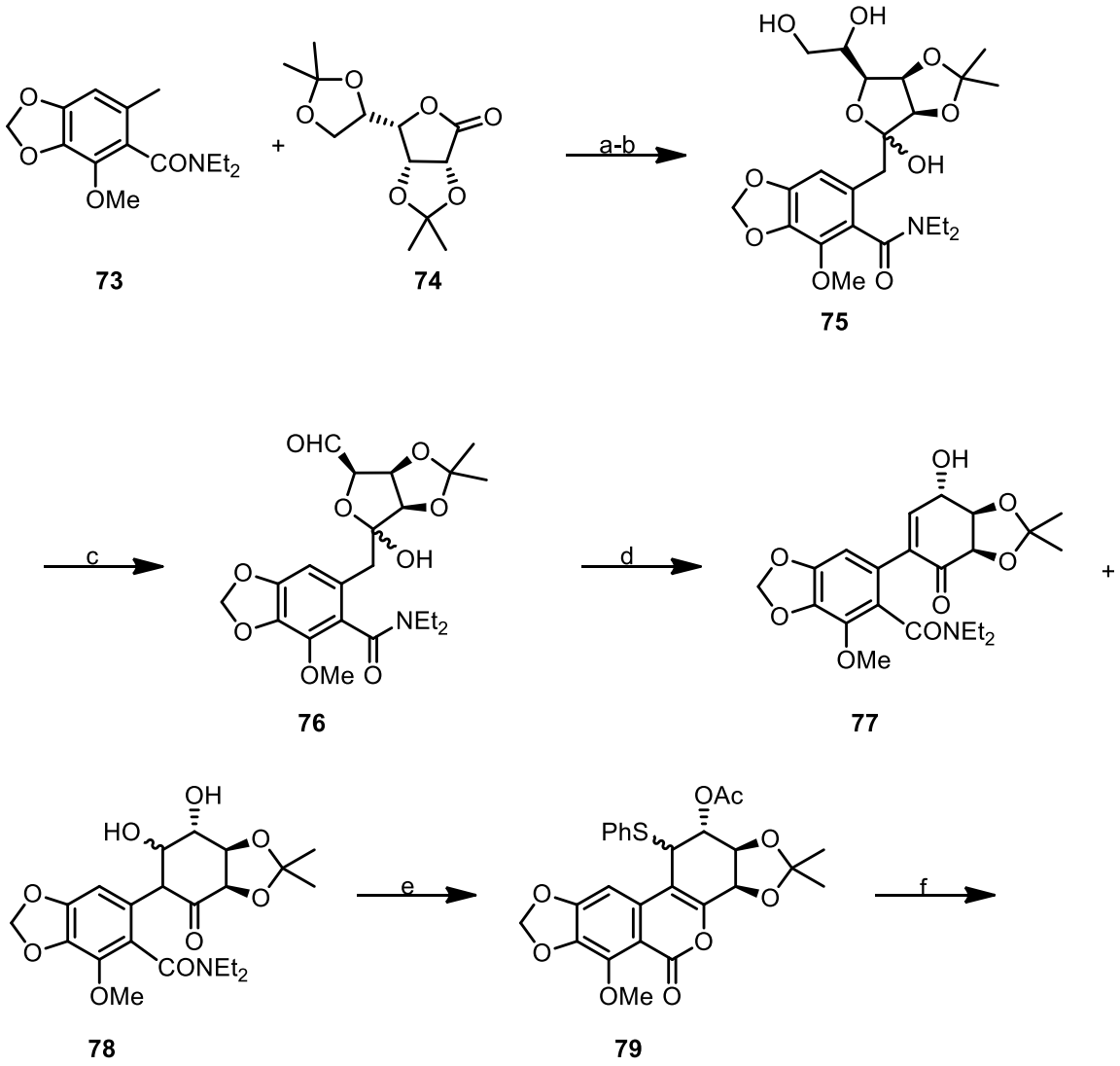
microwave-promoted Overmann rearrangement followed by DIBAL-H reduction was carried out to afford the free amine **71**. Compounds **68** and **71** are the key building blocks of this total synthesis and in order to generate the narciclasine skeleton, Suzuki-Miyaura cross coupling under a microwave irradiation was performed. Global deprotection with TMS-Br afforded *ent*-narciclasine **72**. The total synthesis took 11 steps at an overall yield of 26%.



a) *sec*-BuLi, TMEDA; b) $B(OMe)_3$, H_2O_2, AcOH; c) TBS-Cl, imidazole; d) *sec*-BuLi, TMEDA, I_2
e) Na_2HPO_4, $Me_3O^+BF_4^-$; f) MOMCl; g) pinacolatoborane, $PdCl_2$, dppf; h) *p*-MBDMA, *p*-TsOH; i) OsO_4/NMO; j) MOM-Cl; k)DIBAL-H,MOM-Cl; l) DDQ; m) Cl_3CCN,DBU
n) K_2CO_3, µwave, 165 °C; o) DIBAL-H; p) Pd[0], base, µwave; q) TMS-Br

Scheme 7- Banwell's Total Synthesis of *ent*-narciclasine

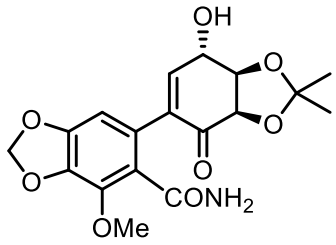
Chapleur and colleagues set out to investigate an efficient approach of the formation of the C-10a-C-10b bond (Scheme 8).[26] In order to achieve this goal, *o*-metalation of the amide **73** reacted with the lactone **74** to form the diols **75**. The conversion of **75** to the aldehyde **76** occurred using of sodium periodate. The cyclization of **76** formed a mixture of elimination product **77** and diol **78**. Both compounds were converted to the desired product **79** by refluxing in thiophenol and trimethylamine. Compound **79** was treated with methanol and ammonia, sodium cyanoborohydride, and formic acid to yield compounds **81**, **82** (25%), **83** (20%), and **84**.



73 **74** **75**

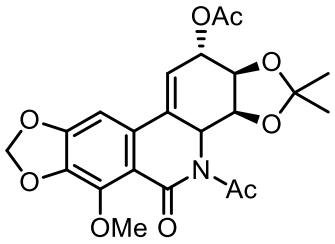
76 **77**

78 **79**

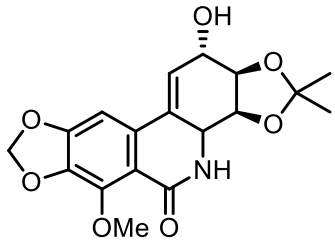


80

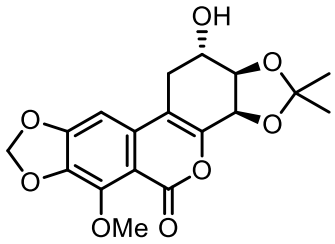
g-i



81



82



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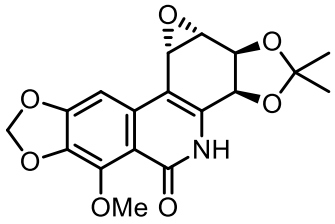
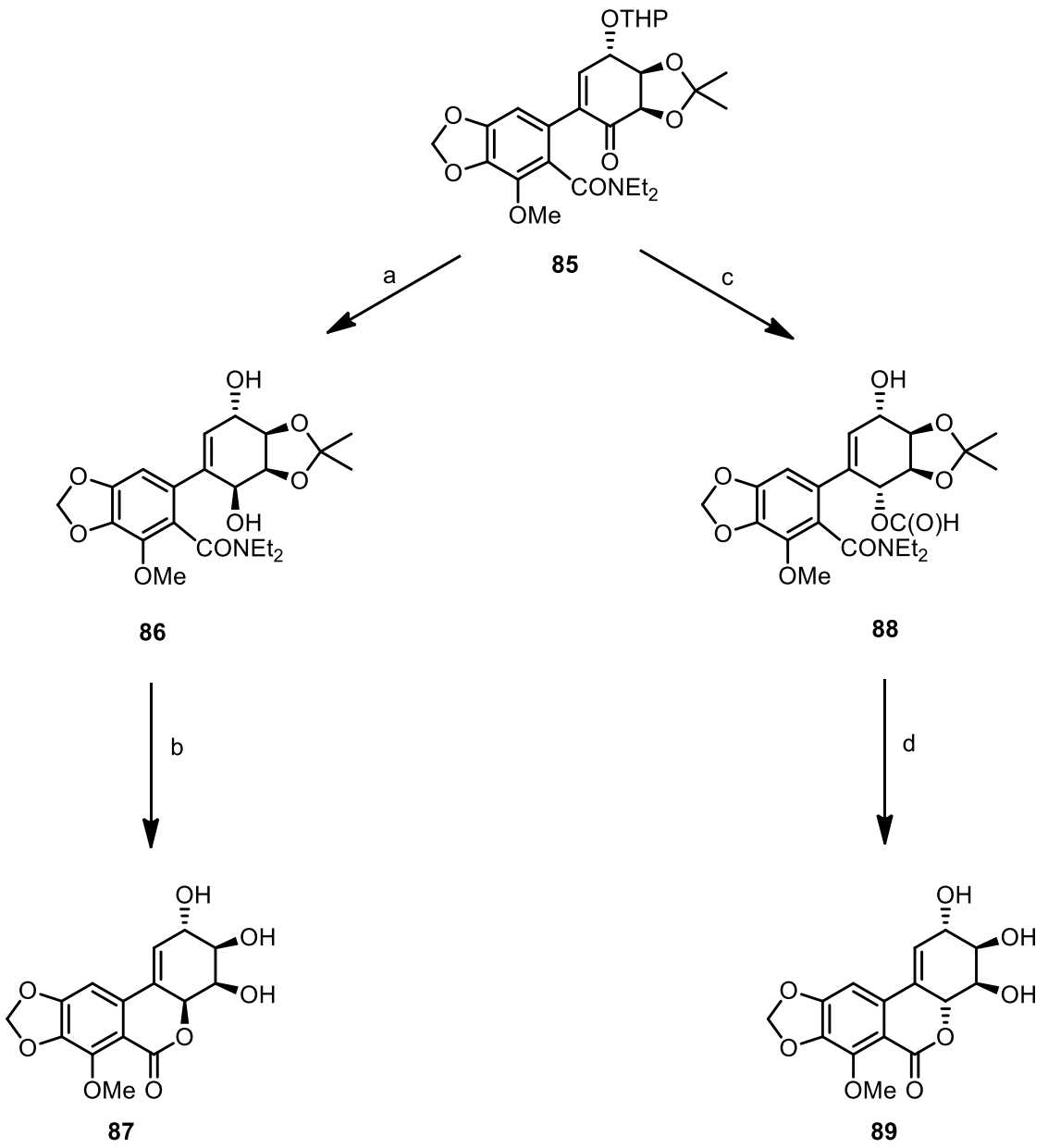


84

a) *sec*-BuLi, THF, -78 °C, 70%; b) AcOH-H_2O, THF, reflux, 90%; c) MeOH, $NaIO_4$, H_2O; d) Na_2CO_3, DBU, 70%; e) Ac_2O, pyridine, 92%; f) PhSH, NEt_3, THF, reflux, 86%; g) NH_3, MeOH; h) $NaBH_3CN$, HCOOH, CH_3CN; i) Ac_2O, DMAP, pyridine

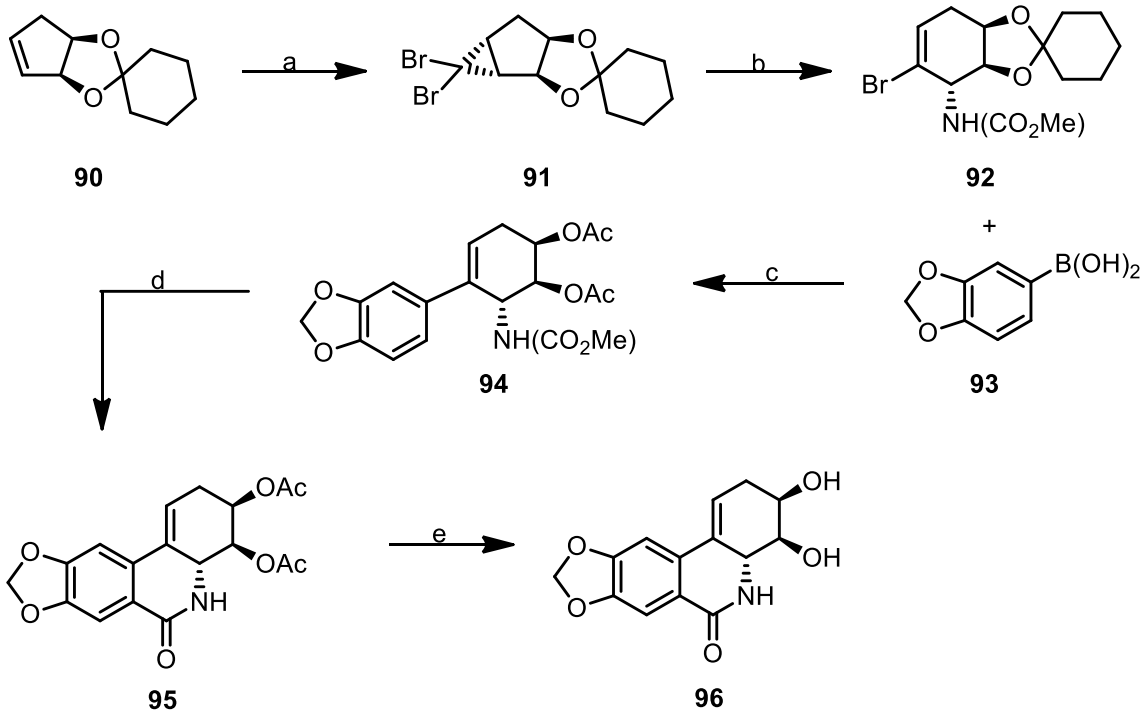
Scheme 8- Chapleur's Narciclasine Analogues



a)i) $NaBH_4$, $CeCl_3$, EtOH, rt; ii) THF, Dowex H^+ resin; b) i) Ac_2O, pyridine, 70 °C; ii) Tf_2O, CH_2Cl_2, pyridine, -30 °C; iii) TFA, THF/H_2O, reflux; iv) MOMCl, iPr_2NEt, CH_2Cl_2, rt; v) NH_3, MeOH, 0 °C then rt; vi) $NaBH_3CN$, HCO_2H, CH_3CN, reflux; c) i) Tf_2O, DMF, -30 °C, 1 h then NaN_3; d) i) NH_2OMe, HCl, THF, reflux; ii) L-Selectride, THF, -30 °C; iii) TFA, THF/H_2O, reflux; iv) MOM-Cl, *i*Pr_2NEt, CH_2Cl_2, rt; v) NH_3, MeOH, 0 °C then rt; vi) $NaBH_3CN$, HCO_2H, CH_3CN, reflux; iv) MeONa, MeOH, rt; AcOH, H_2O 9/1, TFA, reflux.

Scheme 9- Chapleur's Lactone Narciclasine Analogues

In the year 2004, Chapleur prepared more lactone analogues (Scheme 9).[27] Compound **85** was exposed to two different conditions to afford both **86** and **88** with 1:1 mixtures. The cyclization did not occur in basic conditions, therefore acidic conditions were done to deprotect the acetonide and it promoted the wanted product **87** and **88**. These analogues were sent for testing against L1210 cell lines, but did not show any activity.

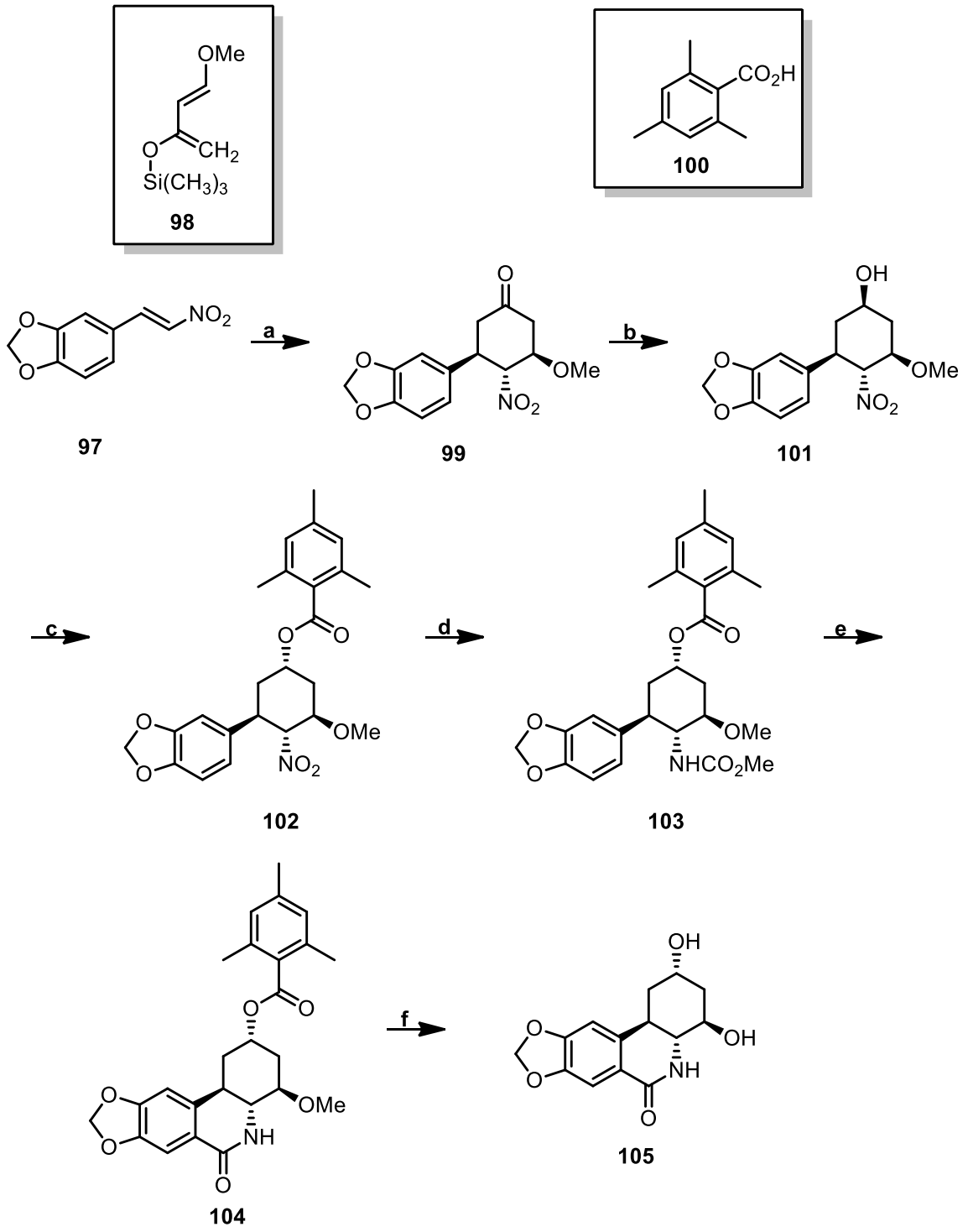


a) $CHBr_3$, 50% aq NaOH, C_6H_6, TEBAC, 0-18 °C; b) i) AgOCN, 1 ,4-dioxane, 100 °C; ii) NaOMe-MeOH 18 °C; c) i) $Pd(PPh_3)_4$, C_6H_6, EtOH; ii) Na_2CO_3 , HCl, THF; d) i) $(MeCO)_2O$, DMAP, C_5C_5N, CH_2Cl_2, 18 °C; ii) Tf_2O, DMAP, CH_2Cl_2, 0-15 °C; iii) HCl, THF; iv) Ac_2O, DMAP, pyridine; e) NaOMe, MeOH, THF

Scheme 10- Banwell's Lycoricidine Analogues

Banwell's synthesis of lycoricidine analogues began with the starting material cyclopentadiene was converted to diols while $Pb(OAc)_4$–mediated oxidation, and transformed into the cyclohexanone acetal **90** (Scheme 10).[25] In order to produce **91**, dibromocarbene reacted with the olefin using Makoza conditions producing the steroselective tricyclic adduct. Silver isocyanate was used for ring opening of **91** to produce **92**, which was isolated using medium pressure liquid chromatography (MPLC). Suzuki cross coupling was performed on the boronic acid **93** and the carbarmate **92** to afford the styrene **94**. The Bischler-Napieralski cyclization was performed in order to obtain the final lycoricidine skeleton. After the deprotection steps **96** was obtained in 8 steps.

McNulty's approach to 3-deoxydihydrolycoricidine began with cycloaddition of nitrostyrene **97** and Danishefsky's diene **98** to provide the cyclohexanone **99**, Scheme 11.[29] The ketone was reduced to alcohol **101** with the use of sodium borohydride and with a modified Mitsunobu protocol **102** was produced. The nitro group was then reduced and converted to the carbamate by aluminum amalgam **103**, which was exposed to Bischler-Napieralski reaction, demethylation, and removal of the ester provided 3-deoxy *trans*-dihydrolycoricidine **105**.



a) **98**, PhMe, 110° C; b) $NaBH_4$, EtOH; c) **100**, $Bu_3P{=}C(CO_2Me)_2$, $PhCH_3$, 70% for 3 steps; d) i) Al(Hg), EtOH, H_2O; ii) $ClCO_2Me$, pyridine, CH_2Cl_2,70%; e)Tf_2O, DMAP, 65%; f) i) BI_3, CH_2Cl_2, 15%; ii) $LiAlH_4$, THF.

Scheme 11- McNulty's 3-deoxydihydrolycoricidine Synthesis

2.1.5. Biological Studies and Anti-cancer Activity of *Amaryllidaceae* Alkaloids

In order for a compound to be classified as a therapeutic, an intense and vigorous process of understanding the pharmacophore must be done. As mentioned earlier, members of the *Amaryllidaceae* family has been used in traditional folk medicine for a wide range of cancer related illnesses. During modern times, Ceriotti[30] was the first to report narciclasine **1** to have antimitotic activity on Sarcoma 180 and inhibition of wheat grain radicles. With the use of anti-cancer assays, murine P-388 lymphocytic leukemia, there was the isolation of a few isocarbostyril *Amaryllidaceae* alkaloids. These alkaloids had low ED_{50} and were found to exhibit activity against a variety of cancer cell lines *in vitro* and *in vivo*.[8] *Amaryllidaceae* alkaloids **1**,**2** and **12-15** displayed antiviral activity *in vitro* against a number of flaviviruses such as Japanese encephalitis, yellow, and dengue fevers.[31] Pancratistatin, narciclasine and 7-deoxypancratistatin display activity *in vivo* with mice infected with Japanese encephalitis; to date few treatments have been discovered for this rare disease.

Table 1- Biological Activity of Selected *Amaryllidaceae* Compounds

Compound	**Mean GI_{50} (μM)**
Narciclasine **1**	0.016
Pancratistatin **2**	0.091
Lycoricidine **12**	0.15
7-deoxypancratistatin **13**	0.100
trans-dihydronarciclasine **14**	0.0126
trans-dihydrolycoricidine **15**	0.0676

The National Cancer Institute (NCI) conducted an in-depth study with standard MTT colorimetric assays with diverse human tumor cell lines for different

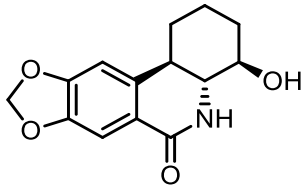
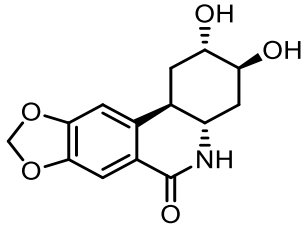
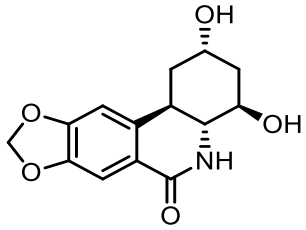
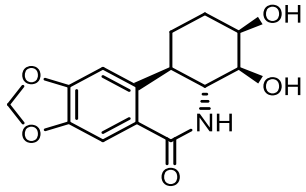
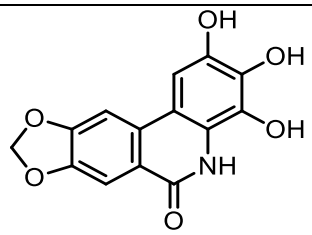
Amaryllidaceae alkaloids. Narciclasine **1**, exhibited potent cytotoxicity against human cell lines and murine P388 leukemia cells (Table 2).[32, 33] In comparison with narciclasine **1** (mean IC_{50}= 0.046 µM), lycoricidine **12** demonstrated cytotoxicity 10 times weaker (mean IC_{50}= 0.33 µM) and pancratistatin **2** was five times weaker (mean IC_{50}= 0.26 µM).[33] Focusing on the Leukemia P388 cell line, dehydronarciclasine **12** has more potent cytotoxicity than narciclasine, but dehydrolycoricidine **13** is at the same level as lycoricidine (Table 2).

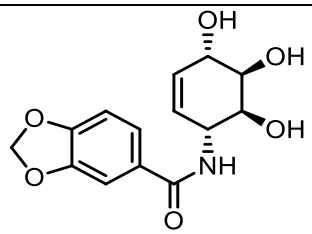
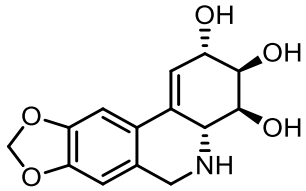
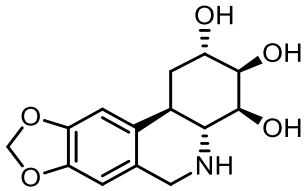
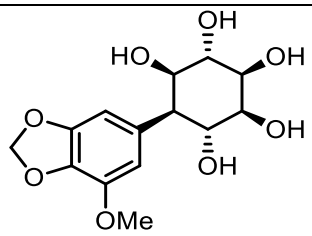
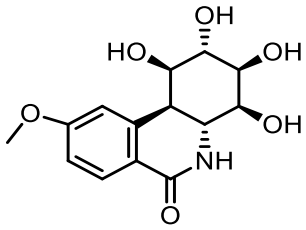
In order to develop successful therapeutics, pharmacology plays an important role. In terms of the C-ring, any removal or alteration of C2-C4 yields compounds that are inactive or possess poor activity (see Table 3). For the B-ring, the amide bond (C6-C5) is crucial; if the N5 is replaced with an oxygen or the nitrogen is removed all activity is lost. Removal of the C7-hydroxyl of the A-ring results in the loss of one order of magnitude in activity. With years of literature precedence in terms of biological activity the only possibility of alternations is C10 and C1 pancratistatin analogues.

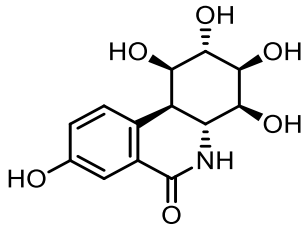
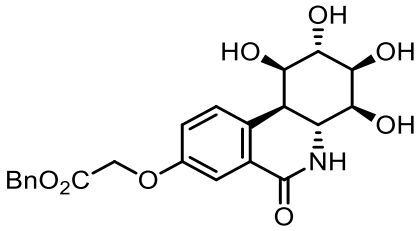
Table 2- *In Vitro* Cytotoxic-Related Antitumor Effects of Naturally Occuring Isocarbostryil Alkaloids (IC_{50} Values in µM)

Compounds	**Cancer Cell Lines**						
	Leukemia P388	Pancreas BXPC-3	Breast MCF-7	CNS SF268	Lung-NSC NCI-H460	Colon KMS0L2	Prostate Du-145
1	0.042	0.011	0.010	0.010	0.027	0.011	0.011
2	0.052	0.061	0.071	0.043	0.098	0.077	0.046
12	0.065	0.24	0.16	0.41	0.18	0.29	0.17
13	1.42	NT	NT	NT	NT	0.71	NT

Table 3- Examples of *Amaryllidaceae* Analogues

Compound	Mean Value of IC_{50} (μM)	Alteration from Lead Structure(**1 & 2)**	Reference
 **106**	Inactive	No hydroxyls on C1-C3, and C7	[34]
 **107**	2.3	No hydroxyls on C1, C4, and C7	[34]
 **108**	Inactive	No hydroxyls C1, C3, and C7	[29]
 **109**	Inactive	No hydroxyls C1, C2, and C7	[35]
 **110**	Inactive	Aromatic C-ring	[28]

 **111**	Inactive	No 10a-10b bond	[36]
 **112**	Inactive	No amide oxygen	[37]
 **113**	5.7	No amide oxygen	[37]
87	Inactive	Oxygen replaces the nitrogen	[27]
88	Inactive	Oxygen replaces the nitrogen	[27]
 **114**	Inactive	No amide fragment opening of the B-ring	[38]
 **115**	12.5	No methylene dioxy bridge	[35]

 **116**	Inactive	No methylene dioxy bridge	[39]
 **117**	Inactive	No methylene dioxy bridge	[39]

Straden *et.al.* did an extensive review on the mechanistic insights to the cytotoxicity of *Amaryllidaceae* alkaloids.[40] Narciclasine was seen inhibiting protein synthesis in rabbit reticulocytes and yeast cell-free systems by halting peptide formation on the ribosome level. It was also shown that narciclasine binds to the peptidyl transferase centre of the 60S ribosomal unit (Figure 7).[41]

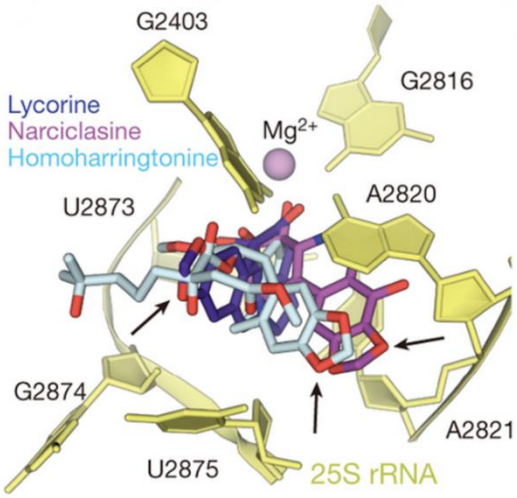
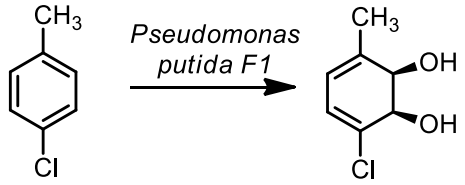


Figure 7- Narciclasine and Lycorine binding to the 60S tRNA P-site[41]

2.2. Aromatic dioxygenases

2.2.1. Discovery of aromatic dioxygenases

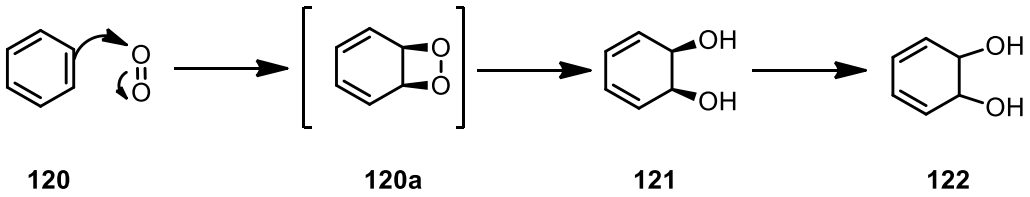
Cyclohexadiene diols are a useful starting material and can be utilized in syntheses of carbohydrates, inositols, alkaloids, and terpenes.[42,43] Synthetic organic chemists are always looking for a process that is not only efficient, but reproducible and the promise of large quantities of a product. In 1968, Gibson studied oxygen fixation and microbial oxidation on aromatic compounds, such as benzene and toluene by *Pseudomonas putida* strain.[44] The rate of metabolism of these aromatic compounds needed to decrease, therefore Gibson exposed *p*-chlorotoluene **118** to the organism grown with toluene to produce the first stable cyclohexadiene diol **119** (Figure 8).[45]



118 **119**

Figure 8- Formation of cis-4-2,3-dihydroxy-1-methyl benzene from p-chlorotoluene with *Pseudomomas putida F1*

The dihydroxylation process was an interest to chemists in order to fully understand the transformation that took place during the fermentation. An experiment was conducted where the fermentation process was carried out in the presence of $^{18}O_2$. Using mass spectroscopy the products were analyzed and the outcome was the material increased by four mass units, which indicated the both oxygen atoms originates from the same oxygen molecule (Figure 9).[46]



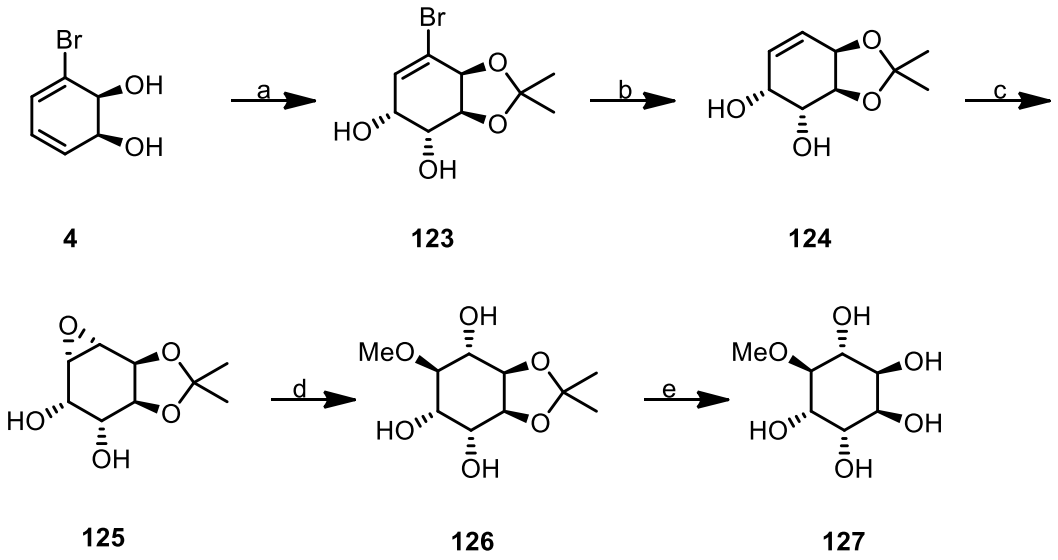
120 **120a** **121** **122**

Figure 9- Proposed Mechansim of diol formation

In 1989 Gibson isolated the genes that were responsible for the toluene dioxygenase enzyme complex, which was cloned and expressed into *Escherichia coli*.[17b] This was beneficial because it is easy to grow and ferment *E. coli* and allow the opportunity for better enzyme concentration because of the incorporation of these plasmids.

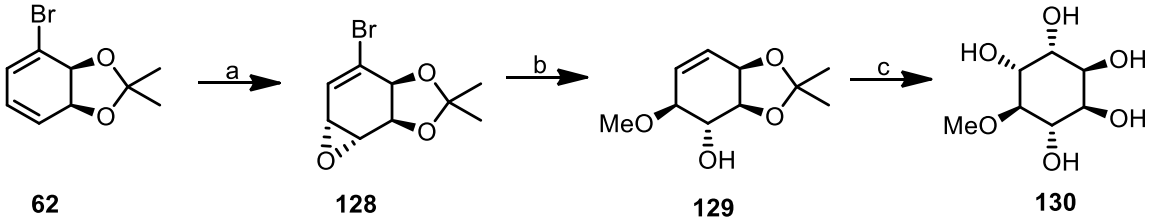
2.2.2. Utilization of diols in the natural product synthesis

The first use of these diols in natural product synthesis was by Ley,[47] but this section will highlight enantiodivergent synthesis of pinitol by Hudlicky which was achieved by using the same bromodiol **4.**[48] Once **4** was protected with an acetonide **62** it was subjected to selective osmylation **123** (Scheme 12) and reductive removal of the bromide **124**. The expoxidation was carried out by using *m*CPBA with **125** followed by the use of methanol and aluminum oxide to yield the methyl ether **126**. The synthesis of (+)-pinitol **127** was achieved in 6 steps. Hudlicky took advantage of the latent symmetry of the diol **62** and was able to carry out the synthesis of (-)-pinitol (Scheme 13) by epoxidation of **62** to obtain **128** and using the same methodology as **127**, (-)-pinitol was also obtained in 6 steps.



a)i) 2,2-DMP, TsOH; ii) OsO_4 , NMO, acetone, H_2O, 85%; b) LAH, THF, 85%; c) *m*CPBA, CH_2Cl_2, 85%; d) MeOH, Al_2O_3 , 90%; e) HCl, H_2O, acetone

Scheme 12- Hudlicky's Synthesis of (+)- Pinitol



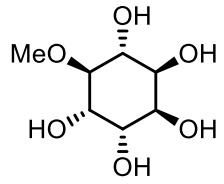
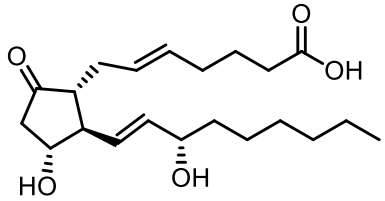
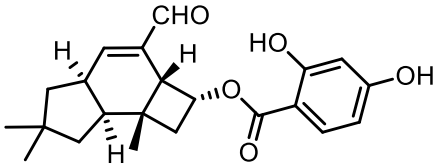
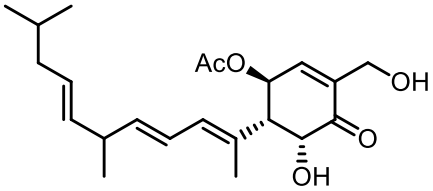
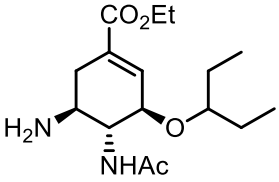
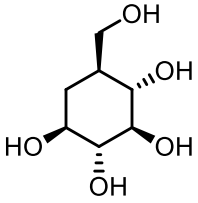
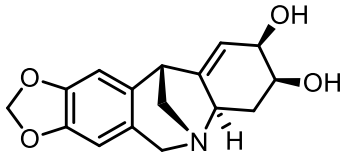
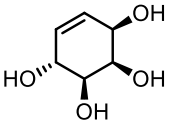
a) *m*CPBA, CH_2Cl_2, 80%; b) i) MeOH, Al_2O_3; ii) LAH, THF, 76%; c) i) OsO_4 , NMO, acetone, H_2O, 85%; ii) HCl, H_2O, acetone, 64%

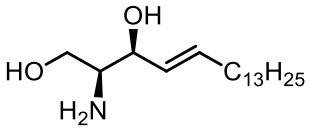
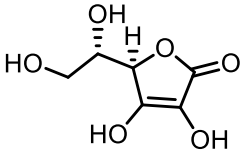
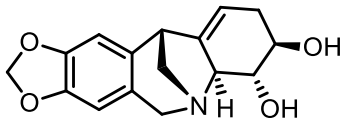
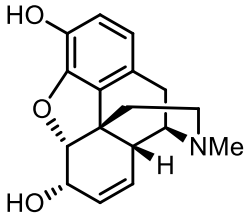
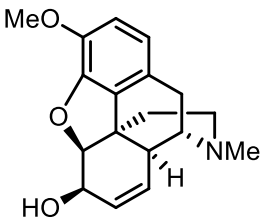
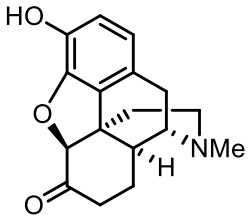
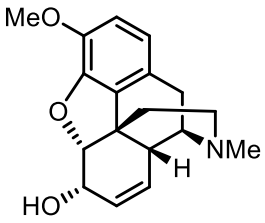
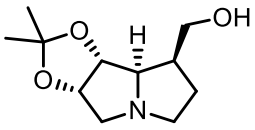
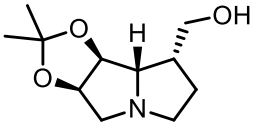
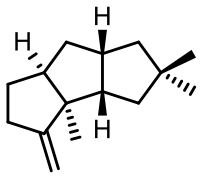
Scheme 13- Hudlicky's Synthesis of (-)-Pinitol

Since the first introduction of *cis*-cyclohexadiene diols these synthons have been used as starting materials in a large number of natural products (Table 4). The diols have allowed the construction of complicated molecules in fewer steps than other simple starting materials. The *cis*-cyclohexadiene diols can be prepared in large quantities and with different functional groups which allows an array of possibilities of what can be

used with. The stereochemistry of these synthons allows the diastereoselective preparation of different classes of natural products.

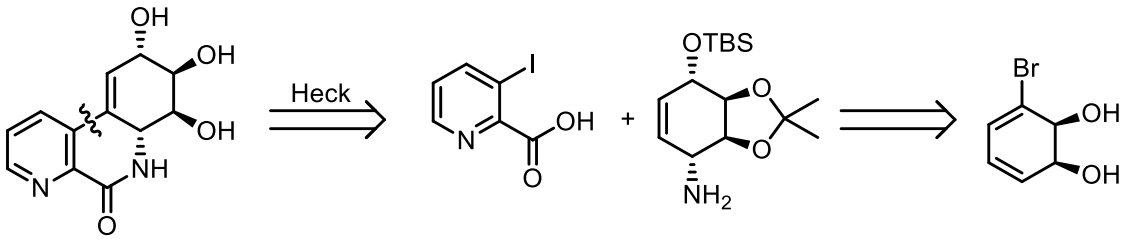
Table 4- Synthetic targets accessed using cyclohexadiene diol

Compound	
 (±)-Pinitol **131**[47]	 Prostaglandin E2 **132**[49]
 (+)-Armillarivin **133**[50]	 (+)-Phorbacin C **134**[51]
 Oseltamivir **135**[52]	 Carba-β -L-glycopyranose **136**[53]
 Brunsvigine **137**[54]	 Conduritol C **138**[55]

 L-*threo*-sphingosine **139**[56]	 Asorbic Acid **140**[57]
 Nangustine **141**[58]	 Morphine **142**[59]
 *ent*-codeine **143**[60]	 *ent*-hydromorphone **144**[61]
 Codeine **145**[62]	 (+)-trihydroxyheliotridane **146**[63]
 (−)-trihydroxyheliotridane **147**[63]	 (−)-hisutene **148**[64]

2.2.3. Synthesis of 10-azanarciclasine

The route for the azanarciclasine series was initially derived for the 7-azanornarciclasine and the same intramolecular Heck approach and identical conduramine **7** can be used for the synthesis of **151** (Figure 10).[65]



149 **150** **7** **4**

Figure 10- Retrosynthesis Analysis of 7-azanornarclasine

The construction of the C-ring was well known from previous research in the group; however finding an approach to the A-ring that is efficient and short was a challenge (Figure 11).[66] It was thought that **153** could be obtained by exposing **156** to halogen dance conditions using a strong base and quenching the anionic intermediate with CO_2. Directed *ortho*-lithiation of **155** and sequential borylation, oxidation and methylation will provide the bromopyridine **154**.

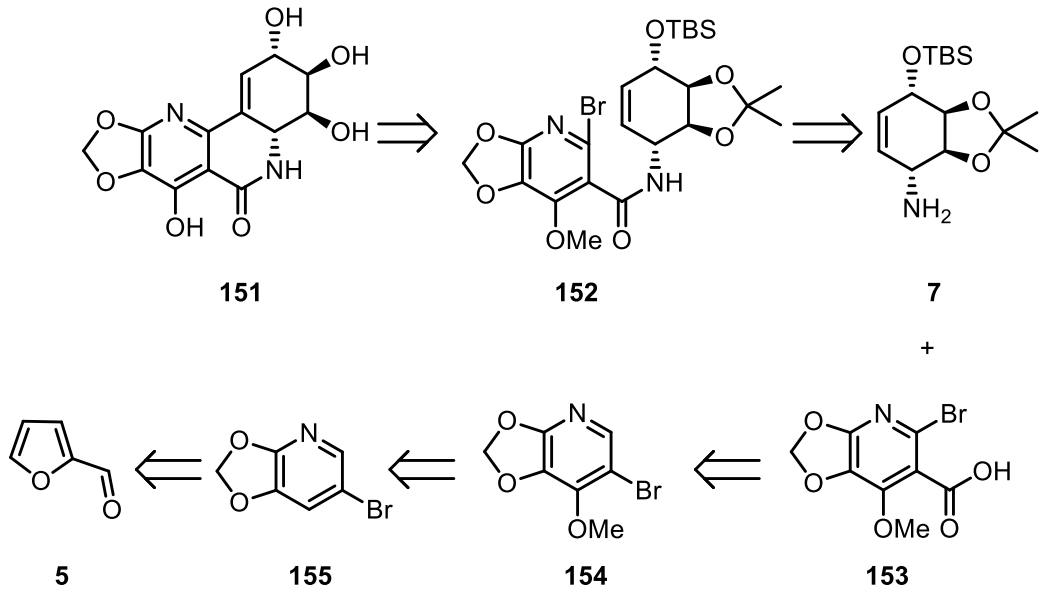
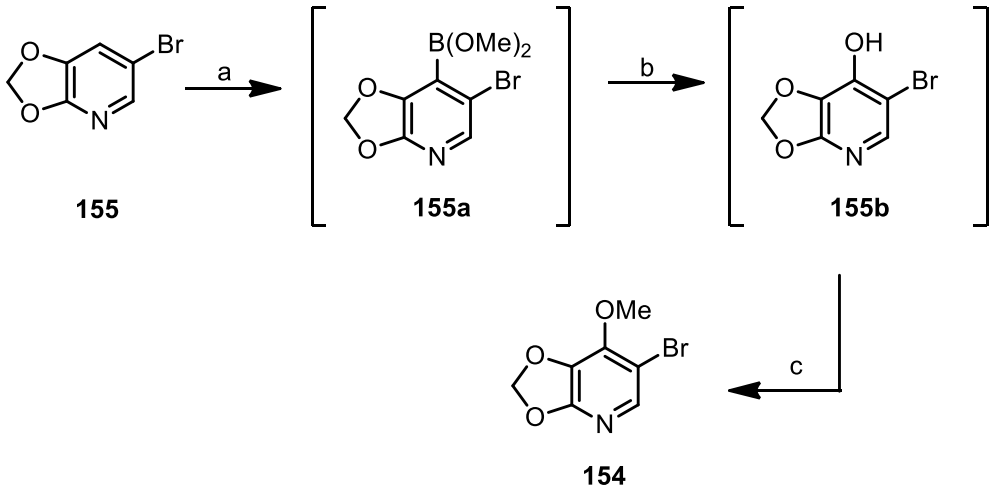


Figure 11- Retrosynthetic Analysis of 10-azanarciclasine

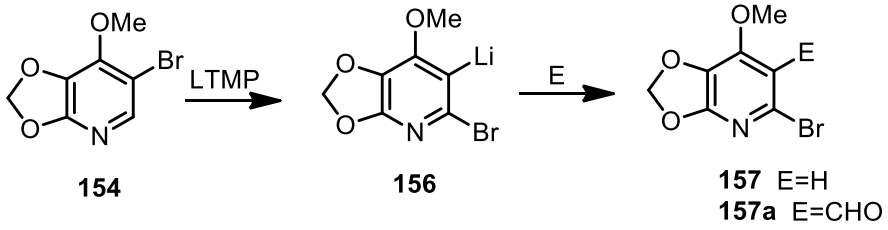
The most efficient method to produce pyridine **154** was directed *ortho*-lithiation of **155** then submitted to borylation with $B(OMe)_3$ and *in situ* oxidation. Compounds **155a** and **155b** were not isolated, but the reactions were monitored by TLC and **154** was obtained after **155b** was exposed to diazomethane. The oxidation step is sensitive to water, therefore it was hypothesized that instead of using the usual oxidant 30% H_2O_2 , due to protodeborylation, urea hydrogen peroxide was a better candidate. Also other methylation agents were used, for example dimethylsulfate, methyl iodide, and Meerwein's salt but only diazomethane proceeded to selectively methylate the 4-pyridinol **155b**.



a) LTMP,THF, -78 °C; b) $B(OMe)_3$; c) i) AcOH, UHP; ii) CH_2N_2, THF, 36%

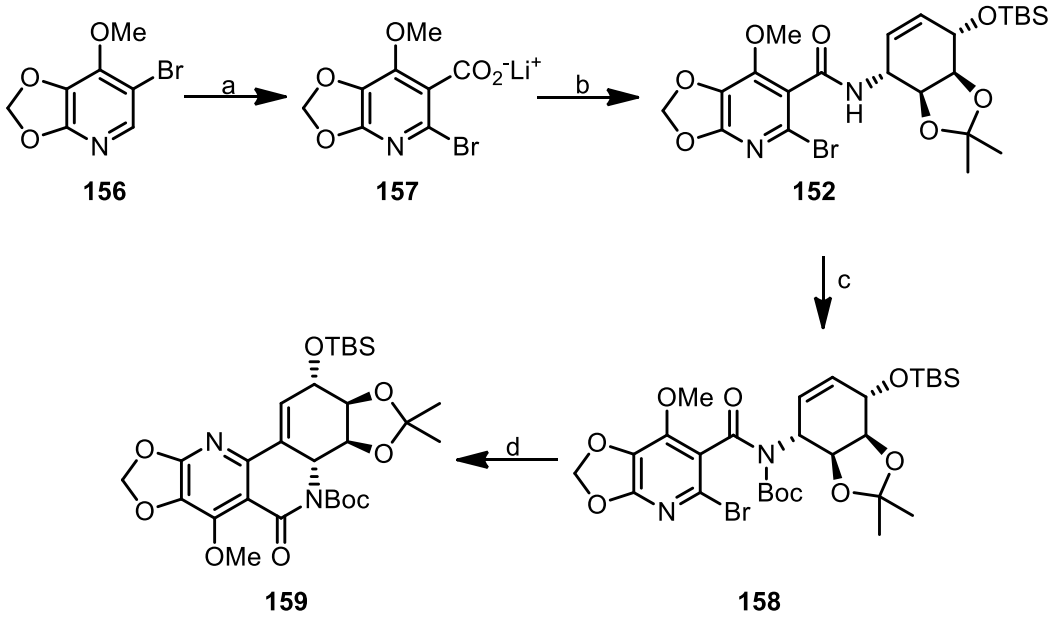
Scheme 14- Synthesis of key intermediate **154**

Having obtained the key intermediate **154**, the uncertain section of the total synthesis was the halogen dance (also known as halogen scramble). In order to achieve halogen dance there are a few key requirements: i) low temperatures; ii) no excess sub-stoichiometric base; iii) the base must be added to the halide; iv) using THF as a solvent; v) a slow reacting electrophile (alkyl halides and DMF).[67] Using these guidelines the aryllithium intermediate **156** was quenched with three electrophiles, methanol **157,** DMF **158a** and CO_2 **157** (Scheme 15). Proton NMR was used to provide sufficient evidence that the halogen dance indeed did occur.



Scheme 15- Halogen Dance Reaction

Once the reaction conditions were established instead of using DMF as an electrophile quench CO_2 was added and lithium carboxylate **157** was not isolated but immediately moved forward to the HBTU coupling **152** (Scheme 16). The intramolecular Heck reaction would only occur if there was a bulky protecting group present on the nitrogen, therefore Boc was added to **152** and left stirring overnight to yield the precursor **158**. Reactions were screened on similar heterocyclic analogues (Table 5) that allowed us to determine which conditions would be appropriate for **158**.[65] The reaction mixture was monitored by TLC and the fully protected 10-azanarciclasine **159** was obtained.



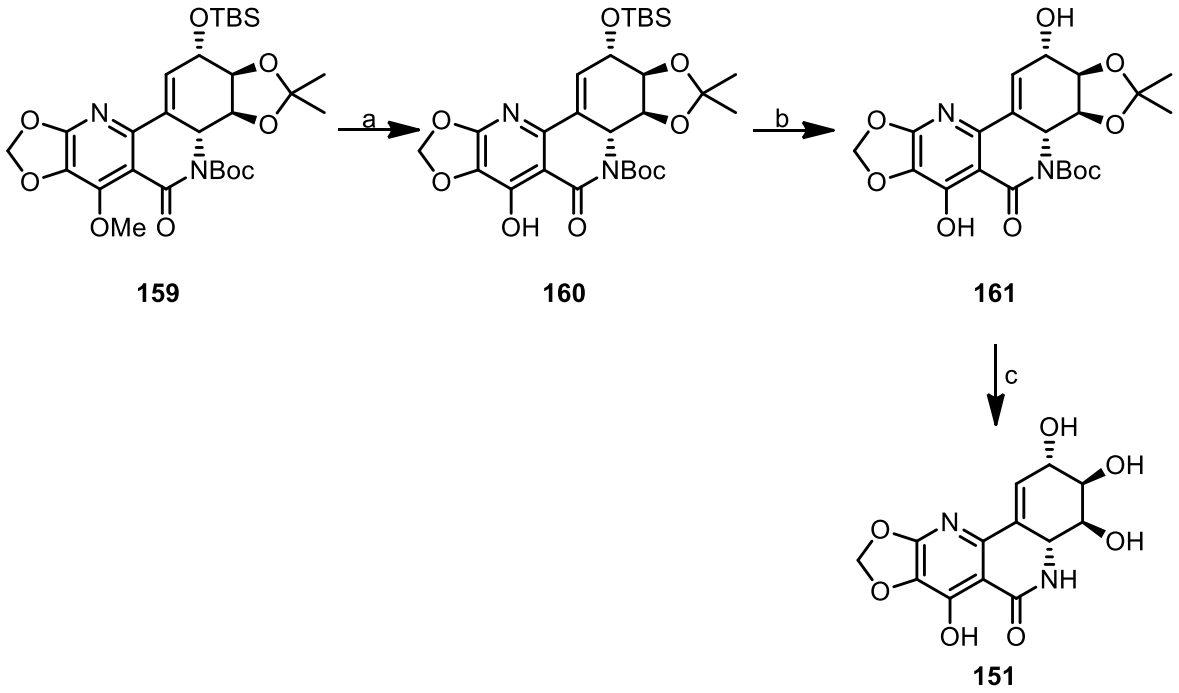
a) i) LTMP, THF, -80°C; ii) CO_2; b) **7**, HBTU, DIPEA, MeCN, 30% for 2 steps; c) Boc_2O, DMAP, MeCN, 80%; d) $Pd(OAc)_2$, dppe, Ag_3PO_4, Cs_2CO_3, PhMe, 90°C, 58%.

Scheme 16- Synthesis of Fully Functionalized Skeleton of 10-azanarciclasine

Conditions	Outcome
$Pd(dppf)_2Cl_2$, dppf, Ag_3PO_4 dioxane, 60°C, 24 h	No reaction
$Pd(OAc)_2$, dppe, Ag_3PO_4, Et_3N toluene, 80°C, 18 h	Traces of product
$Pd(OAc)_2$, PPh_3, Et_3N, $AgNO_3$, MeCN, 24 h, r.t to 80°C	No reaction
$Pd(OAc)_2$, dppe, $AgNO_3$, Cs_2CO_3, toluene, 24 h, 110°C	Mixture
$Pd(OAc)_2$, BINAP, Ag_3PO_4, Et_3N toluene, 80°C, 18 h	No reaction

Table 5- Screening for Intramolecular Heck for **149**

The deprotection of sequence for **159** is described in Scheme 17. Demethylation of the pyridinol was brought about through the use of lithium chloride at high temperatures to afford **160**. Once the product was isolated it was subjected to standard desilylation conditions in the presence of TBAF to yield alcohol **161**. Different deprotection conditions were investigated to remove the acetonide and Boc groups. HCl in methanol led to decomposition of the material, but when the weaker formic or trifluoroacetic acid, there was no deprotection. To solve this problem wet trifluoroacetic acid (H_2O 5% v/v) was used to obtain 10-azanarciclasine **151** in 11 steps (9 one-pot reactions).



159 **160** **161** **151**

a) LiCl, DMF, 100°C, 56%; b) TBAF, THF, 90%; c) TFA, H_2O, CH_2Cl_2, 77%.

Scheme 17- Deprotection of 10-azanarciclasine

3. Discussion

3.1.1. Synthesis of 7-aza-10-methoxy-narciclasine

Natural products have been a significant and convenient source for new therapeutics. *Amaryllidaceae* alkaloids, in particular the isocarbostyril family, are known to be compounds with high activity, with the possibility to be worthwhile drug candidates. The concerns associated with this class of compounds is the limited availability from natural sources and the meagre solubility profiles. Although the issues of solubility have been addressed maintaining activity is still a problem, therefore the generation of these derivatives still relies on the supply of natural sources. The main objective of this project is to produce a new class of heteroanalogues that resolve the

problem of solubility and activity, through an efficient and divergent route to narciclasine. In order to achieve this goal a common, abundant starting material must be chosen and a synthetic plan that has the least amount of steps possible.

The major theme of this discussion will be the utilization of the bromodiol **4** for the approach to the total synthesis of 7-azanarciclasine **9**. Despite the two analogues having the same starting materials, a divergent strategy was applied to arrive at the two isomeric products.

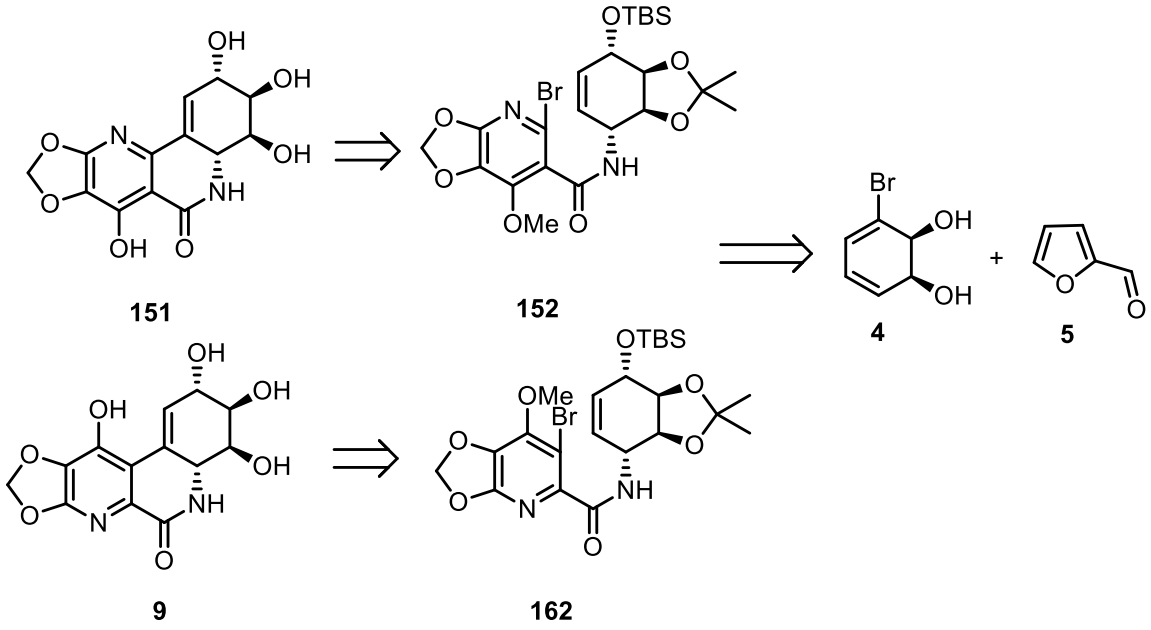
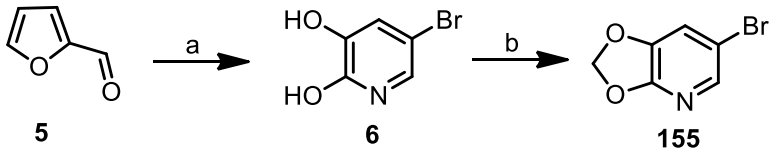


Figure 12- Synthetic Strategy for 10-azanarciclasine and 7-azanarciclasine

Our attention turned to using the same methodology described in the historical of 10-azanarciclasine to synthesize 7-azanarciclasine **9** (Figure 12). The unique portion of the synthesis of the 10 and 7-aza series was the transformation of furfural **5** to the pyridine diol **6**, using a procedure published by Dallacker (Scheme 18).[68] The yield for

the installation of the methylene bridge in **155** is surprisingly low, but when compared to literature examples it is seen to be around 20% one can assume the nitrogen hinders the reaction unlike having a simple aromatic ring. The reaction mechanism for this transformation is unknown, therefore we proposed a mechanism (Figure 13). Electrophilic addition of Br_2 occurs at the more electron rich alkene of the furan ring **5**. Lone pair assisted elimination of bromide forms a cation which is attacked by H_2O **III**. Acid catalyzed ring opening results in an enol which eliminates the second bromide to yield cis alkene **VI**. A second bromination yield the dialdehyde **VIII**, which reacts with sulfamic acid to give bis-aminal **IX**, which eliminates water to yield enamine **XI**. Elimination of bromide via the enamine and tautormization yields **XIII**, which upon loss of SO_3 yields the desired product **6**.



5 **6** **155**

a) i) 1 eq. Br_2, H_2O, 0 °C; ii) HCl, H_2O, 0 °C; iii) 1 eq. Br_2, 0 °C; iv) H_2NSO_3H, 50 °C, 65%, b) CH_2Br_2, K_2CO_3, CuO, DMF, 95 °C 18%.

Scheme 18- Synthesis of intermediate **155**

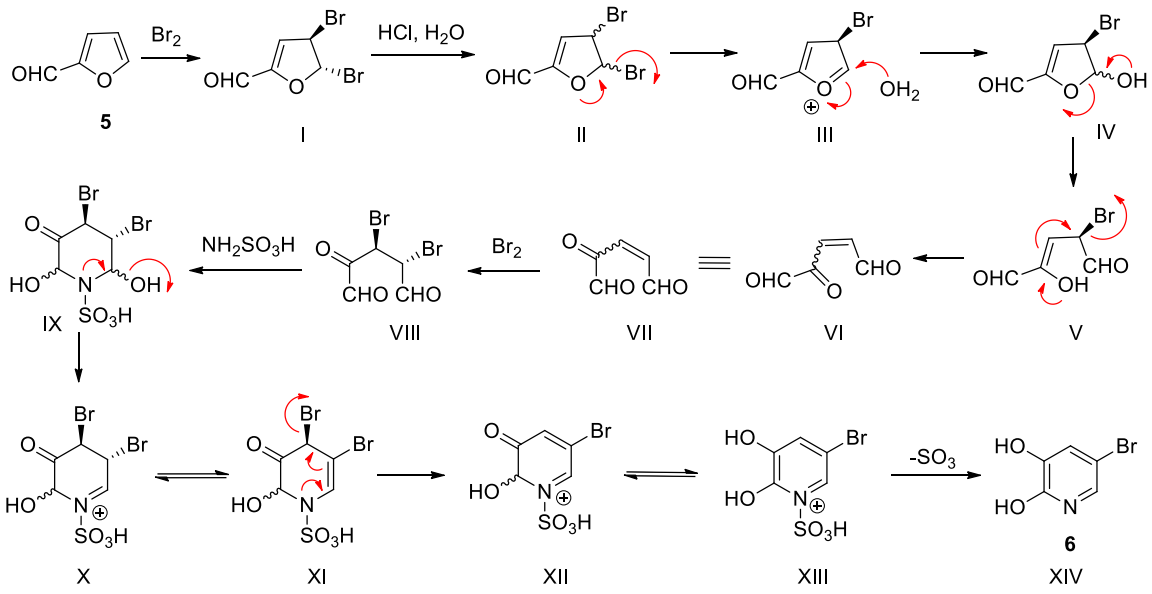
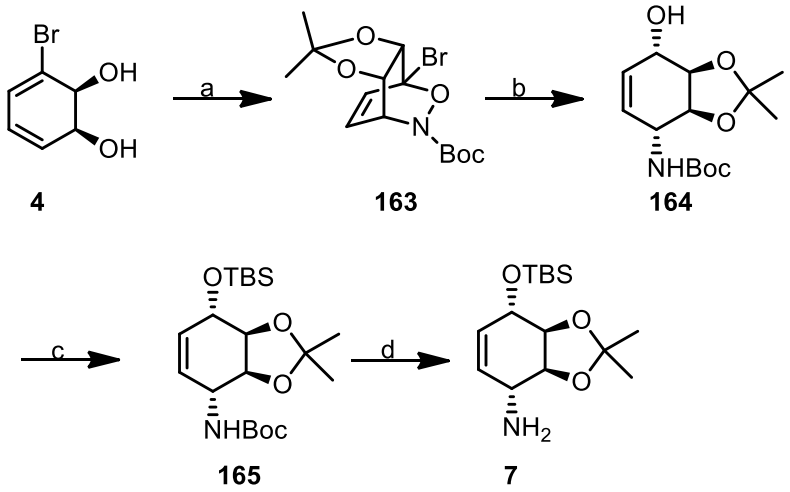


Figure 13- The Hayward-Hudlicky Modification of the Vshyvenko Hypothesis

The formation of oxazines is well known in the Hudlicky group, therefore the synthesis of the C-ring in principle was quite simple (Scheme 19). The bromodiol **4** reacted with BocNHOH using $NaIO_4$ as an oxidant in a hetero-Diels-Alder type reaction to yield **163**. Aluminum amalgam was prepared and selectively cleaved the C-Br and N-O bonds to form **164**. The hydroxyl was protected with TBS **165** and the Boc was cleaved using TFA and the amine **7** for coupling.



a) BocNHOH **169**, $NaIO_4$, 0°C to rt, 74%; b) Al(Hg), THF/H_2O, 89%; c) TBS-Cl, imidazole, CH_2Cl_2, 81%; d) TFA, CH_2Cl_2, 0°C, 86%.

Scheme 19- Construction of the C-ring

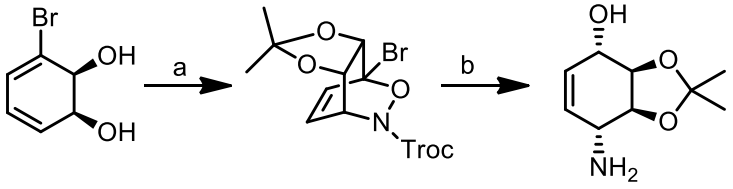
During the synthesis of 10-azanarciclasine the deprotection of Boc **165** was often problematic in terms of selectivity between the three acid-labile protecting groups. Selective deprotection of the Boc group was only observed on rare occasions despite strict efforts to maintain the conditions. The different reaction conditions done were summarized in Table 6. In order to obtain consistency with this system the final decision was made to change the protecting group on the oxazine.

Table 6- Screening of Deprotection Conditions

Reaction Conditions	Outcome
TFA, 0°C, CH_2Cl_2 , 20 min	Removal of acetonide
TFA, 2,2-DMP,0°C, 4Å Sieves, CH_2Cl_2	Starting Material
TFA:CH_2Cl_2 (1:4) , 20 min	Mixture of product (**7**) & no acetonide
Silica gel, CH_2Cl_2, H_2O, 140°C, 24 h	No reaction
TMSOTf, NEt3, CH_2Cl_2 , 0°C to rt	No reaction

Scheme 20 outlines the new synthetic route for the C-ring. Although it eliminated the problem of selective de-protection new issues arose. The production of

the oxazine **166** was performed as before, BocNHOH replaced by TrocNHOH. This structure was interesting to us and was sent for crystal structure to prove the stereochemistry (Figure 13). Using aluminum amalgam was attractive considering in literature Troc had the potential to eliminate using electrode Al(Hg) at −1.7V, thus would result in the acetonide-protected conduramine in one cleavage step. Our hypothesis was proven to be correct and using proton NMR it was determined that Troc was being eliminated to produce the desired product **49**. Table 7 summarizes different reduction conditions that have been tried. It was observed **49** was not stable, therefore all precautions were taken by deactivating the silica with NH_4OH during the purification process. Unfortunately the impurity associated with compound **49** still can be seen on TLC.



4 **166** **49**

a) TrocNHOH **170**, $NaIO_4$, 0°C to rt, 72%; b) Al(Hg), THF/H_2O, 30%

Scheme 20- Synthesis of the Oxazine

Table 7-Reduction of **166**

Reaction Conditions	Outcome
SmI_2, HMPA, rt, 8 h	No reaction
Al(Hg), THF/H_2O, 2 h	30% product
Zn, MeOH	No reaction

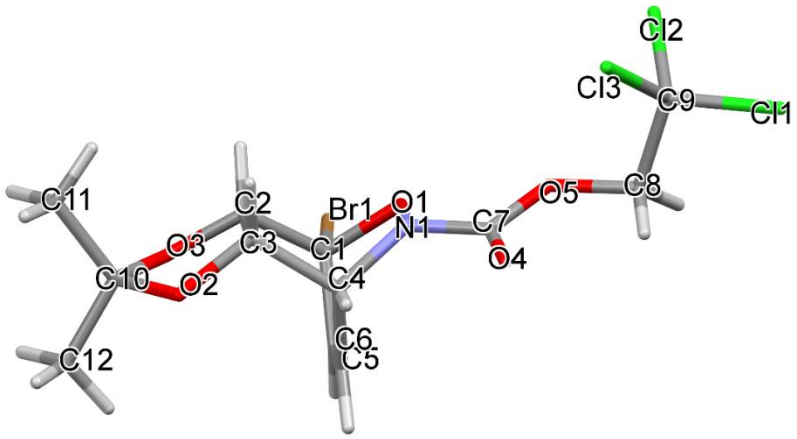
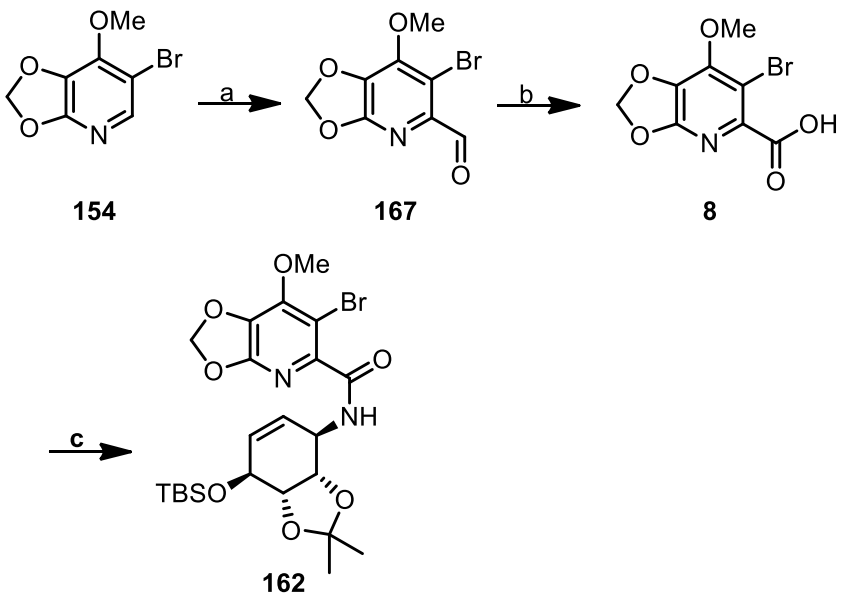


Figure 13- Crystal Structure of **166**

There is literature precedent that discusses the difficulty of fully functionalizing a pyridine ring due to the nitrogen, therefore finding an efficient synthetic route was a problem.[69] For 10-azanarciclasine, the carboxylate needs to be installed in the 3-position and in-situ the amide coupling reaction was carried out. For this alkaloid, the same route was taken by blowing in CO_2 through a drying agent or adding in dry ice pellets, but the carboxylate did not form in the

2- position of the pyridine ring. A different route was taken where after the lithiation the reaction was quenched with DMF **167**, Pinnick oxidation was done using Fukuyama conditions to yield **8**.[70] The purification of the carboxylic acid was trying, recrystallizing with a few different solvents was attempted but was not successful. The purification of **8** using column chromatography was attempted, but unfortunately decomposed on the column. Due to the problems of reproducibility of **7** and stability of **49** the total synthesis of 7-azanarciclasine could not be achieved.

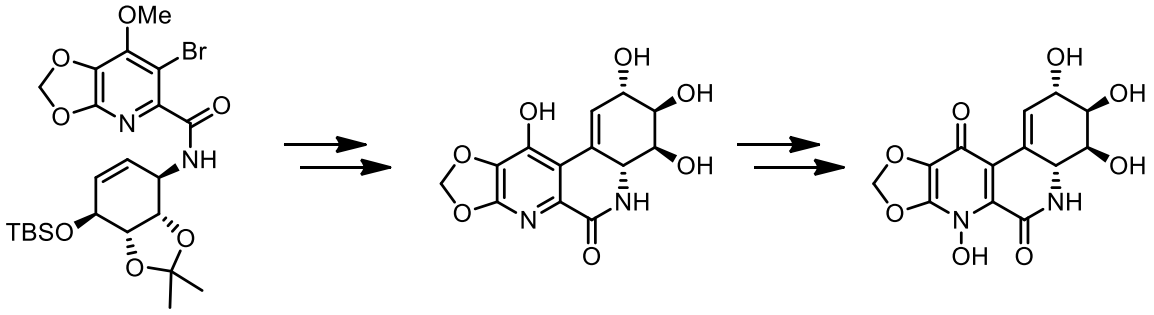


a) LTMP, DMF, -78°C, 52% ; b) $NaClO_2$, NaH_2PO_4 , 2-methyl-2-butene, *t*-BuOH, H_2O, 80%; c) **7**, HBTU, DIPEA, MeCN, 45%.

Scheme 21- Synthetic Route to the A-ring and Coupling

4. Conclusion and Future Works

The successful synthesis of 10-azanarciclasine orchestrated by Dr. Sergey Vshyvenko for this family of compounds, and solubility measurements validate our analog design concept. However, the biological activity of this analog was decreased from the parent compound, which led us to undertake the synthesis of 7-azanarciclasine. Unfortunately this synthesis has not reached completion because of the different regiochemistry of the pyridine ring and the difficulties of the reliability of selective deprotection of the conduramine C-ring. This has led to us exploring different protection strategies for this ring system, which should lead to the successful synthesis of 7-azanarciclasine and its N-oxide analogues.



162 **9** **168**

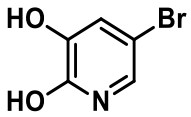
5. Experimental

5.1. General Experimental Details

Reactions were carried out under inert atmosphere in flame-dried glassware unless stated otherwise. Solvents were distilled prior to use: CH_2Cl_2 from CaH_2, DMF from CaH_2, and THF from Na/benzophenone. Thin layer chromatography was performed with pre-coated silica gel aluminum sheets (EMD silica gel 60 F254), detection by UV and with "CAM" solution (5 g of $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1 g of $Ce(SO_4)_2$, 100 mL of 10% H_2SO_4) or 0.5% aqueous $KMnO_4$ solution followed by heating. Flash chromatography was performed using silica gel SiliaFlash P60 or F60 from Silicycle (40–66 μm). Optical rotation was measured in a 1-dm cell at 20-25 ˚C and 589 nm, concentration *c* in g/100 mL. IR spectra were collected on a Bruker Alpha IR spectrometer equipped with a Platinum ATR module. ^1H NMR and ^{13}C NMR spectra were recorded at Bruker Avance I 300, Bruker Avance III HD 400, and Bruker Avance I 600 and 75 MHz, 100MHz, and 150 MHz, respectively, and were calibrated on the solvent residual peak ($CDCl_3$ = 7.26 ppm; CD_3OD= 3.31), the chemical shifts are reported in ppm. Data are reported as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad; coupling constants (*J*) in Hz, integration.

5.2. Detailed Experimental Procedures

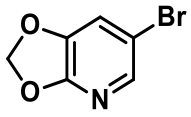
2,3-Dihydroxy-5-Bromopyridine (6).



Freshly distilled furfural (24.4 mL, 254 mmol) and crushed ice (175 g) were stirred and cooled to 0-2 °C. Bromine (13 mL, 255.5 mmol) was added dropwise *via* an addition funnel over 40 min. concentrated HCl (37%, 21 mL) was added and the reaction mixture stirred for 30 min maintaining the temperature at 0 °C , after which a second portion of bromine (13 mL, 255.5 mmol) was added dropwise. Once the reaction was complete the mixture was filtered and the straw yellow filtrate was transferred into a flask. Sulfamic acid (37.5 g, 386 mmol) was added and the mixture was heated to 50 °C for 1.5 h. The reaction mixture was filtered and the solid transferred to a crystallization dish, treated with distilled water (5 mL) and dried in the oven overnight. The product was dissolved in AcOH (400 mL) ,charcoal pellets were added and the mixture was heated to 40 °C for 3 h, after which the charcoal was removed by filtration and the filtrate was allowed to cool down to room temperature, placed in an ice bath, and crystals was collected. The product was obtained as grey crystals (52.3 g, 68 %).

6: mp 245 °C (AcOH), [lit.[68] 249 °C (AcOH)

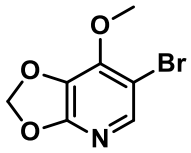
6-Bromo-[1,3]dioxolo[4,5-b]pyridine (155).



2,3-Dihydroxy-5-Bromopyridine **6** (25.9 g, 0.137 mol), DMF (40 mL), K_2CO_3 (47.3 g, 0.343 mol), copper(II) oxide (3.29 g, 0.0411 mol), and dibromomethane (19.14 mL, 0.274 mol) were combined and left stirring under argon for 15 min. The reaction mixture was heated to 95 °C for 16 h under an inert atmosphere. The mixture was filtered and the filtrate was collected and diluted with H_2O (500 mL); a white precipitate was formed and second filtration was conducted. The precipitate was discarded and the filtrate collected was extracted with EtOAc (3 x 200 mL), the combined organic layers were concentrated under reduced pressure. The product was purified by flash column chromatography (hexanes/EtOAc 4:1) affording **155** a white solid (5.22 g, 25%).

155: R_f 0.7 (hexanes/EtOAc 2:1); mp = 67-69 °C (EtOH), [lit.[66] 69-71° C]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.71 (s, 1 H), 7.12 (s, 1 H), 6.09 (s, 2H) ^{1}H NMR matched with lit. value.

6-Bromo-7-methoxy-[1,3]dioxolo [4,5-b]pyridine (154).

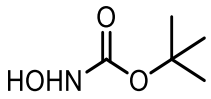


To a solution of 2,2,6,6-tetramethylpiperidine (0.64 mL, 3.8 mmol) in THF (5 mL) at -60°C *n*-BuLi (1.5 mL, 3.29 mmol) was added dropwise. Reaction mixture was stirred for 15 min at -40 °C, then cooled down to -80 °C and a solution of 6-Bromo-[1,3]dioxolo[4,5-

b]pyridine (0.52 g, 2.5 mmol) in THF (5 mL) was added dropwise, while maintaining the temperature at -80 °C. The reaction mixture was stirred for an additional 40 min, followed by addition of $B(OMe)_3$ (0.70 mL, 6.25 mmol) and the reaction mixture was warmed up to 0 °C. The reaction mixture was then cooled to -60 °C and AcOH (0.43 mL, 7.5mmol) was added, followed by addition of urea hydrogen peroxide (0.71 g, 7.5 mmol) and stirred overnight at rt. Excess of urea hydrogen peroxide was quenched by $NaHSO_3$ (sat. aq.), extracted with EtOAc (3×20 mL). The combined organic layers were dried over Na_2SO_4 and concentrated under reduced pressure. The residue was redissolved in THF:MeOH mixture (10:1, 30 mL) at 0 °C. The ethereal solution of diazomethane was added until the disappearance of starting material was observed by TLC monitoring. The reaction mixture was evaporated and the residue was purified by flash column chromatography (hexane/EtOAc 4:1) to yield 0.2 g of **154** (34%) as a yellow crystalline compound.

154:R_f 0.45 (hexanes/EtOAc 5:1); mp 117-119 °C ($CHCl_3$) [lit.[66] 69-71° C ($CHCl_3$)]; ^{1}H NMR (300 MHz, CDCl3) δ 7.72 (s, 0.75 H), 7.61 (s, 0.2 H), 6.08 (s, 2H), 4.19 (s, 3H); ^{1}H NMR matched with literature.

***t*-Butyl hydroxycarbamate (169).**

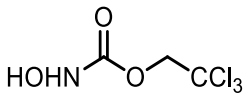


Hydroxylamine hydrochloride (9.6 g, 0.14 mol) and potassium carbonate (7.2 g, 0.069 mol) in Et_2O (60 mL) and H_2O (4 mL) was stirred for 4 h at room temperature with

evolution of CO_2. A solution of di-*t*-butyl carbonate (20 g, 0.092 mol) in Et_2O (40 mL) was added dropwise at 0 °C and the reaction mixture was stirred at room temperature for 7 h. The organic phase was decanted and the solids were washed with Et_2O and the organic solutions were combined and evaporated under reduced pressure. The compound was then recrystallized from pentane and toluene providing **169** as white crystals (8.1 g, 89%).

169: mp 52-55 °C (pentane-tolune), [lit.[71] 58-59 °C (cyclohexane- tolune)];

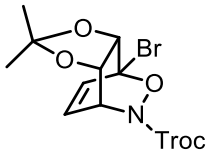
2,2,2-trichloroethyl hydroxycarbamate (170).



Hydroxylamine hydrochloride (13.9 g, 0.200 mol) was added to a 1.5 M NaOH (160 mL, 0.240 mol). The solution was cooled to 0 °C and 2,2,2-trichloroethyl chloroformate (5.30 mL, 38.5 mmol) was added dropwise. The reaction mixture was stirred at room temperature for 2 h and acidified to pH 5 with conc. HCl. The resulting mixture was extracted with ether (5 x 200 mL), the combined organic layers were washed with brine (100 mL), then dried over $MgSO_4$ and evaporated under reduced pressure. The crude product was then recrystallized with chloroform and hexanes to afford colourless crystals (5.85 g, 73%).

170:R_f 0.1 (Hexanes/EtOAc 1:1); mp 88-91 °C (chloroform-hexanes), [lit.[72] 87-88 °C (benzene-light petroleum)];

(3aS,4R,7R,7aS)-2,2,2-trichloroethyl 4-bromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[d][1,3]dioxole-8-carboxylate (166).

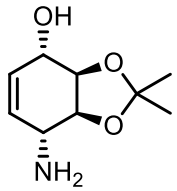


To a solution of diol **4** (4.61 g, 24.2 mmol) in 2,2-dimethoxypropane (25 mL) was added a catalytic amount of *p*-toluenesulfonic acid in a flask. To improve the solubility of the reaction mixture, dichloromethane (25 mL) was added. To complete the consumption of starting material, the solution was cooled to 0 °C and after 70 min 10 mL of water was added. $NaIO_4$ (5.62 g, 26.4 mmol) was added to the reaction vessel before **176** (5.53g, 26.5 mmol) in 50 mL of methanol was added dropwise. After addition, the solution was warmed up to room temperature and stirred for 16 h. Upon completion of the reaction, excess of saturated aqueous sodium bisulfite was added until a white color was obtained. The mixture was extracted with EtOAc (5 × 200 mL), the combined organic phase was washed with brine (2 × 15 mL) and dried over Na_2SO_4, and the solvent was removed *in vacuo*. The reaction product was purified by suction column chromatography[73] (Hexanes/EtOAc 9:1,4:1, and 1:1) affording 7.27 g (72%) as a yellow solid.

166: R_f = 0.4 [(hexanes/EtOAc (1:1)]; mp 128-130 °C (CH_2Cl_2-pentane); $[\alpha]^{20}_D$ -1.51 (*c* = 1, $CHCl_3$); IR (neat) ν 2989, 2955, 2934, 1711, 1435, 1413, 1381, 1343, cm^{-1}; ^1H NMR (600 MHz, CDCl3) δ 6.52 (d, *J* = 7.93 Hz, 1H), 6.45 (t, *J* = 7.17 Hz, 1H), 5.11 (s, 1H),4.85 (d, *J* = 12.09 Hz ,1H), 4.72 (d, *J* = 11.33 Hz, 1H), 4.66 (s, 2H), 1.37 (s, 3H), 1.34 (s, 3H) ; ^{13}C NMR (75 MHz, CDCl3) δ 155.9, 134.2, 131.5, 111.8, 94.7, 87.5, 81.3, 75.5, 74.1, 53.6, 25.7,

25.5; HRMS (ESI) calcd for $C_{12}H_{14}BrCl_3NO_5$ $[M+H]^+$ 435.9121:Found 435.9125; Anal. Calcd for $C_{12}H_{13}BrCl_3NO_5$: C, 32.94; H, 3.00. Found C (33.08); H (2.77)

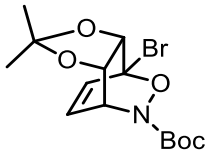
(3a*R*,4*S*,7*R*,7a*S*)-7-amino-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-ol (49).



To a solution of **158** (1.08 g, 2.49 mmol) in THF:H_2O (40:5 mL) was added aluminum amalgam, prepared by dipping small square strips of Al foil (3.203 g, 31.4 mmol) sequentially to NaOH (1 M), distilled water, $HgCl_2$ (0.5% solution), distilled water, and THF. The reaction mixture was stirred for 1 h and was filtered through sand and celite, washed with methanol (3 × 80 mL). The filtrate was concentrated, the residue was dissolved in toluene and concentrated for the second time. The product was isolated by flash column chromatography (CH_2Cl_2/MeOH/NH_4OH 80:20:1) affording 0.118 g (26%) of **49** as colourless viscous oil.

49: R_f = 0.4 [(CH_2Cl_2/MeOH (4:1)]; $[\alpha]_D^{20}$ =+3.45 (c = 1.18 , MeOH), [lit.[14] $[\alpha]_D^{20}$ = +11.2]; ^{1}H NMR (400 MHz, MeOD) δ 5.80-5.75 (m, 1H), 5.67-5.64 (m, 1H),4.11-4.09 (m,2H), 3.95-3.90 (dd, *J* =12.22, 5.58 , 1H), 3.22 (m, 1H), 1.44 (s, 3H), 1.36 (s, 3H);

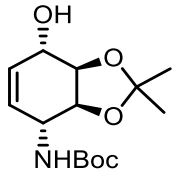
(3a*S*,4*R*,7*R*,7a*S*)-tert-Butyl 4-bromo-2,2-dimethyl-3a,4,7,7a-tetrahydro-4,7-(epoxyimino)benzo[d][1,3]dioxole-8-carboxylate (163).



To a solution of diol **4** (3.5 g, 18.3 mmol) in 2,2-dimethoxypropane (10 mL) was added a catalytic amount of *p*-toluenesulfonic acid in a flask. To improve the solubility of the reaction mixture, in acetone (5 mL). The solution was cooled to 0 °C and after 15 min H_2O (7 mL) was added followed by $NaIO_4$ (5.52 g, 25.8 mmol) in one portion, after which a solution of *t*-butyl hydroxycarbamate (2.9 g, 0.022 mol) in MeOH (40 mL) was added dropwise. After addition was complete, the solution was allowed to warm to room temperature and stirred for 16 h. Excess of saturated aqueous sodium bisulfite was added carefully until a light straw color was obtained. The mixture was extracted with Et_2O (3 × 100 mL), the combined organic phase was washed with brine (2 × 15 mL) and dried over Na_2SO_4, and the reaction mixture was concentrated under reduced pressure. The product was purified by flash column chromatography (hexanes/EtOAc 5:1) affording **163** a colourless solid (4.91 g, 74%)

163: R_f 0.6 (hexanes/EtOAc 2:1); mp = 150 °C (EtOH), [lit.[66]155 °C (EtOH)]; $[\alpha]_D^{20}$ = +2.0 (c=2.5, $CHCl_3$), [lit.[66] $[\alpha]_D^{20}$ = +25.3 (*c* = 1.0, $CHCl_3$)]; ^{1}H NMR (400 MHz, $CDCl_3$) δ 6.50 (d, *J* =8.1 Hz, 1H), 6.40-6.38 (dd, *J* = 4.0, 3.5 Hz, 1H), 4.99-4.97 (m,1H), 4.61 (s, 2H), 1.47 (s, 9H), 1.36 (s, 3H), 1.33 (s,3H) ^{1}H NMR matched with literature.

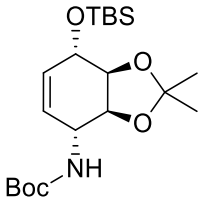
***t*-Butyl ((3aS,4R,7S,7aR)-7-hydroxy-2,2-dimethyl-3a,4,7,7a tetrahydrobenzo[d][1,3]dioxol-4-yl)carbamate (164).**



To a solution of **163** (7.04 g, 19.4 mmol) in THF:H_2O (140:14 mL) was added, aluminum amalgam was prepared by dipping small square strips of Al foil (4.94 g, 92.9 mmol) sequentially to NaOH (1 M), distilled water, $HgCl_2$ (0.5% solution), distilled water, and THF. The reaction mixture was stirred overnight and then filtered through sand and celite, washed by methanol (3 × 220 mL). The filtrate was concentrated, the crude product was dissolved in toluene and concentrated for the second time. The product was isolated by flash column chromatography (hexanes/EtOAc 4:1) affording 4.93 g (89%) of **164** as colourless viscous oil.

164:R_f 0.4 (hexanes/EtOAc 2:1); $[\alpha]_D^{20}$ = -1.2 (c=1.0, $CHCl_3$) [lit.[74] $[\alpha]_D^{20}$ = -41(*c* = 1.0, $CHCl_3$)];^{1}H NMR (300 MHz, $CDCl_3$) δ 5.92 (m, 1H), 5.80 (m, 1H), 5.04 (d, *J* = 7.0 Hz, 1H), 4.23-20 (dt, *J* = 16.9, 6.0 Hz, 3H), 4.04 (m, 1H), 2.22 (s, 1H), 1.45 (s, 12H), 1.35 (s, 3H).^{1}H NMR matched with literature.

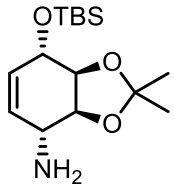
***tert*-Butyl ((3a*S*,4*R*,7*S*,7a*S*)-7-{[*tert*-butyl(dimethyl)silyl]oxy}-2,2-dimethyl-3a,4,7,7a-tetrahydro-1,3-benzodioxol-4-yl)carbamate (165).**



To a solution of **164** (1.94 g, 6.79 mmol) in CH_2Cl_2 (20 mL) was added imidazole (0.695g, 10.2 mmol) followed by TBSCl (1.13 g, 7.48 mmol) at room temperat. After overnight stirring, the reaction mixture was quenched with water (35 mL), extracted with CH_2Cl_2 (3 x 20 mL). The combined organic layers was dried with Na_2SO_4 and the solvent removed under reduced pressure. The product was purified by flash column chromatography (hexanes/EtOAc 8:1) affording 0.425 g (70%) of **165** as a white solid.

165: R_f = 0.9 (hexanes/EtOAc 6:1); mp 102-105 °C ($CHCl_3$); $[\alpha]_D^{20}$ = -2 (*c*=1.0, $CHCl_3$) [lit.[74] $[\alpha]_D^{20}$ =-0.8 (*c* = 1.0, $CHCl_3$),]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.06-6.01 (m, 1H), 5.35 (d, *J* = 8.7 Hz, 1H), 4.32-4.28 (m, 3H), 4.19-4.16 (m, 1H), 1.48 (s, 9H), 1.37 (s, 3H), 1.31 (s, 3H), 0.90 (s, 9H) 0.13 (s, 3H), 0.09 (s, 3H); ^{1}H NMR matched with literature.[66]

(3aS,4R,7S,7aS)-7-((tert-butyldimethylsilyl)oxy)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[d][1,3]dioxol-4-amine (7).

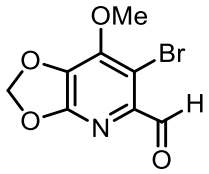


Trifluoroacetic acid (1.3 mL) was added dropwise to a solution of **165** (0.0497 g, 0.125 mmol) in CH_2Cl_2 (5.2 mL) at 0°C. After 10 min the reaction was neutralised by the

addition of conc. ammonia (2 mL). The organic layer was extracted with CH_2Cl_2 (3 × 5 mL), washed with brine (5 mL) and was evaporated. The free amine **7**, 0.0364 g (80%), was obtained as a yellow oil and the sample was purified by flash column chromatography (Hexanes/EtOAc 1:1).

7:R_f 0.4 (hexanes/EtOAc 1:1); $[\alpha]_D^{20}$ = -9.9 (*c*=1.0, $CHCl_3$) [lit.[66] $[\alpha]_D^{20}$ =+15.5 (*c* = 1.0, $CHCl_3$)]; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.73 (dt, *J* = 9.8, 2.3 Hz, 1H), 5.65 (dt, *J* = 9.8, 2.3 Hz, 1H), 4.19-4.16 (m, 1H), 4.11 (dd, *J* = 7.8, 4.8 Hz, 1H), 3.89 (dd, *J* = 7.5, 6.3 Hz, 1H), 3.28 (dd, *J* = 5.7, 2.1 Hz, 1H), 1.66 (s, 2H), 1.44 (s, 3H), 1.34 (s, 3H), 0.91 (s, 9H) 0.12 (s, 3H), 0.11 (s, 3H) ^{1}H NMR matched with literature.[66]

6-Bromo-7-methoxy-[1,3]dioxolo[4.5-b]pyridine-5-carbaldehyde (167).

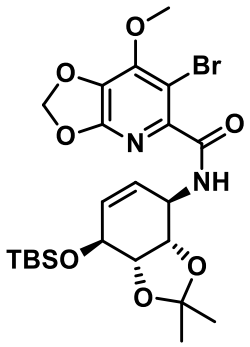


A solution of 2,3,5,6-tetramethylpiperidine (0.33 mL, 1.94 mmol) in dry THF (15 mL) was cooled to −70 °C and *n*-BuLi (0.84 mL, 1.94 mmol) was added dropwise and the reaction mixture was warmed up to −30 °C. The reaction was then cooled to −70 °C and a solution of 6-bromo-7-methoxy-[1,3] dioxolo [4,5-b] pyridine (0.15 g, 0.65 mmol) dissolved in dry THF (2 mL) added dropwise over 30 min. After 30 min of stirring at −70 °C, dry DMF (0.3 mL, 1.94 mmol) was added to the reaction mixture and kept at a constant temperature (-75 °C). The reaction was quenched with saturated $NH_4Cl_{(aq)}$ and the mixture was extracted with EtOAc (3 × 10mL). The combined organic extracts were

then dried by Na_2SO_4 and the solvent evaporated under reduced pressure. The product was purified by flash column chromatography on silica gel (Hex/EtOAc 3:1) affording a lavender solid (0.0871 g, 52%).

167: $R_{f=}$ 0.3 [(hexanes/EtOAc (2:1)]; mp 156 °C (MeOH); IR ($CHCl_3$) ν 3583, 3349, 2961, 2920, 1605, 1488, 1441, 1429, 1388, 1350, 1282, 1211, 1149, 1101, 1053, 1006, 961, 918, 855, 772, 740, 666, 502, 471, 455 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 10.13 (s, 1H), 6.15 (s, 2H), 4.23 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 188.7, 159.5, 145.1, 140.1, 130.6, 112.9, 101.3, 60.1; MS (+EI) *m*/*z* 259 ([M, $^{79}Br]^+$, 100), 261 ([M, $^{81}Br]^+$, 98), 258 (18), 233 (50), 231 (54), 215 (22), 186 (67), 132 (35); HRMS (ESI) calcd for 259.9558; found (259.9548);

6-bromo-*N*-((3a*S*,4*R*,7*S*,7a*S*)-7-(*tert*-butyldimethylsilyloxy)-2,2-dimethyl-3a,4,7,7a-tetrahydrobenzo[*d*][1,3]dioxol-4-yl)-7-methoxy-[1,3]dioxolo[4,5-*b*]pyridine-5-carboxamide (162).



Aldehyde **167** (0.0230 g, 0.088 mmol) was dissolved in *t*-BuOH (3 mL) and H_20 (1 mL). Sodium phosphate monobasic (0.0182 g, 0.132 mmol) and 2-methyl-2-butene (0.05 mL, 0.44 mmol) are added to the reaction mixture and is cooled to 0 °C. Once reaching target temperature sodium chlorite (0.024 g, 0.27 mmol) is added and stirred for half an hour before removing the ice bath. After three hours starting material is completely

consumed and crude product was moved forward to the next reaction. Carboyxlic acid **8** (0.0083 g, 0.030 mmol) was dissolved in dry MeCN (1.5 mL) , HBTU (0.013g, 0.033 mmol) was added, followed by DIPEA (0.01 mL, 0.060 mmol) and solution of amine **7** (0.0089, 0.030 mmol) in MeCN (1.5 mL). Reaction mixture was stirred for five hours, quenched with NH_4Cl (sat. aq., 2 mL) extracted with EtOAc (3 × 3 mL). Combined organic layer was dried over Na_2SO_4, evaporated subjected to flash column chromatography (silica gel, CH_2Cl_2/ MeOH 20:1) to yield 0.0075g (45%) of **162** as a beige crystalline compound.

162*:*R_f 0.6 (CH_2Cl_2/ MeOH 20:1); mp 68-71°C ($CHCl_3$); $[\alpha]_D^{20}$ =-15.2 (*c* = 1, MeOH); IR (neat, cm^{-1}) ν 1608, 1506, 1461, 1370, 1257, 1210, 1103, 1059, 960 ;^{1}H NMR (400 MHz, $CDCl_3$) δ 7.61 (d, *J* = 8.9 Hz, 1H), 6.07 (s, 2H), 6.00 (dd, *J* = 9.7, 4.6 Hz, 1H), 5.92 (dd, *J* = 9.7, 4.6 Hz, 1H), 4.69-4.67 (m, 1H), 4.37 – 4.35 (m, 1H), 4.27-4.24 (m, 2.25H), 4.20 (s, 3.35H), 1.41 (s, 3H), 1.31 (s, 3H), 1.31 (s, 3H), 0.86 (s, 9H), 0.11 (s, 3H), 0.10 (s, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 163.59, 157.73, 146.45, 140.06, 132.89, 129.64, 128.82, 108.65, 106.76, 101.06, 68.61, 60.16, 47.94, 26.86, 25.96, 24.76, 18.30, -4.58, -4.66; ; MS (+EI) *m/z* (%) 421(7), 258 (13), 180 (63), 167 (28), 75 (94), 73 (47), 71(41), 69 (35), 57 (100), 55 (50) ; HRMS (+EI) calcd for $C_{22}H_{30}BrN_2O_7Si$[M-C_4H_9, Br-81]:501.0517 Found: 501.0519.

6. Appendix

Table 8- Crystal Data and Structure Refinement for **166**

Identification code	zgii68_0m_b	
Empirical formula	C12 H13 Br Cl3 N O5	
Formula weight	437.49	
Temperature	173(2) K	
Wavelength	0.71073 Å	
Crystal system	Monoclinic	
Space group	$P2_1$	
Unit cell dimensions	a = 8.6267(3) Å	α= 90°.
	b = 6.9749(2) Å	β= 102.987(2)°.
	c = 13.9151(4) Å	γ = 90°.
Volume	815.86(4) $Å^3$	
Z	2	
Density (calculated)	1.781 Mg/m^3	
Absorption coefficient	3.030 mm^{-1}	
F(000)	436	
Crystal size	0.219 x 0.167 x 0.064 mm^3	
Theta range for data collection	2.423 to 30.645°.	
Index ranges	-12<=h<=12, -9<=k<=9, -19<=l<=19	
Reflections collected	23778	
Independent reflections	4969 [R(int) = 0.0309]	
Completeness to theta = 25.242°	99.9 %	
Absorption correction	Numerical	
Max. and min. transmission	0.8676 and 0.6237	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	4969 / 1 / 200	
Goodness-of-fit on F^2	1.023	
Final R indices [I>2sigma(I)]	R1 = 0.0243, wR2 = 0.0514	
R indices (all data)	R1 = 0.0333, wR2 = 0.0532	
Absolute structure parameter	0.020(7)	
Extinction coefficient	n/a	
Largest diff. peak and hole	0.552 and -0.423 $e.Å^{-3}$	

Table 9- Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3)for **166**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
Br(1)	2878(1)	6000(1)	4013(1)	22(1)
Cl(1)	9924(1)	6119(2)	9519(1)	40(1)
Cl(2)	7341(1)	3714(1)	8500(1)	33(1)
Cl(3)	10376(1)	3508(1)	7996(1)	32(1)
O(1)	5891(2)	5551(3)	5018(1)	21(1)
O(2)	7523(2)	4782(3)	2514(1)	27(1)
O(3)	4854(2)	4621(3)	2371(1)	24(1)
O(4)	9864(2)	6564(3)	6087(2)	33(1)
O(5)	7672(2)	6079(4)	6708(1)	21(1)
N(1)	7581(2)	5546(3)	5104(2)	21(1)
C(1)	5108(2)	5996(5)	3997(2)	16(1)
C(2)	5517(3)	4332(4)	3386(2)	18(1)
C(3)	7335(4)	4385(4)	3486(2)	21(1)
C(4)	8038(3)	6029(7)	4169(2)	23(1)
C(5)	7252(4)	7891(4)	3793(2)	23(1)
C(6)	5693(4)	7872(4)	3701(2)	20(1)
C(7)	8486(3)	6158(5)	5979(2)	21(1)
C(8)	8531(4)	6746(4)	7652(2)	26(1)
C(9)	9016(3)	5078(4)	8370(2)	24(1)
C(10)	6080(3)	4247(5)	1847(2)	27(1)
C(11)	6102(4)	2142(6)	1594(3)	43(1)
C(12)	5832(4)	5532(6)	973(2)	42(1)

Table 10- Bond lengths [Å] and angles [°] for **166**

Br(1)-C(1)	1.929(2)
Cl(1)-C(9)	1.771(3)
Cl(2)-C(9)	1.773(3)
Cl(3)-C(9)	1.767(3)
O(1)-N(1)	1.436(3)
O(1)-C(1)	1.463(3)
O(2)-C(10)	1.425(3)
O(2)-C(3)	1.425(3)
O(3)-C(2)	1.415(3)
O(3)-C(10)	1.437(3)
O(4)-C(7)	1.198(3)
O(5)-C(7)	1.358(3)
O(5)-C(8)	1.434(3)
N(1)-C(7)	1.360(3)
N(1)-C(4)	1.480(3)
C(1)-C(6)	1.493(4)
C(1)-C(2)	1.525(4)
C(2)-C(3)	1.544(4)
C(2)-H(2A)	1.0000
C(3)-C(4)	1.526(5)
C(3)-H(3A)	1.0000
C(4)-C(5)	1.503(5)
C(4)-H(4A)	1.0000
C(5)-C(6)	1.322(4)
C(5)-H(5A)	0.9500
C(6)-H(6A)	0.9500
C(8)-C(9)	1.528(4)
C(8)-H(8A)	0.9900
C(8)-H(8B)	0.9900
C(10)-C(12)	1.488(4)
C(10)-C(11)	1.511(5)
C(11)-H(11A)	0.9800
C(11)-H(11B)	0.9800

C(11)-H(11C)	0.9800
C(12)-H(12A)	0.9800
C(12)-H(12B)	0.9800
C(12)-H(12C)	0.9800

N(1)-O(1)-C(1)	108.60(16)
C(10)-O(2)-C(3)	107.3(2)
C(2)-O(3)-C(10)	107.6(2)
C(7)-O(5)-C(8)	115.1(2)
C(7)-N(1)-O(1)	116.19(19)
C(7)-N(1)-C(4)	120.2(2)
O(1)-N(1)-C(4)	113.14(18)
O(1)-C(1)-C(6)	110.1(2)
O(1)-C(1)-C(2)	105.4(2)
C(6)-C(1)-C(2)	112.22(19)
O(1)-C(1)-Br(1)	103.46(13)
C(6)-C(1)-Br(1)	113.6(2)
C(2)-C(1)-Br(1)	111.3(2)
O(3)-C(2)-C(1)	110.9(2)
O(3)-C(2)-C(3)	105.1(2)
C(1)-C(2)-C(3)	106.8(2)
O(3)-C(2)-H(2A)	111.3
C(1)-C(2)-H(2A)	111.3
C(3)-C(2)-H(2A)	111.3
O(2)-C(3)-C(4)	109.4(2)
O(2)-C(3)-C(2)	104.4(2)
C(4)-C(3)-C(2)	109.1(2)
O(2)-C(3)-H(3A)	111.2
C(4)-C(3)-H(3A)	111.2
C(2)-C(3)-H(3A)	111.2
N(1)-C(4)-C(5)	108.3(2)
N(1)-C(4)-C(3)	103.3(3)
C(5)-C(4)-C(3)	110.3(2)
N(1)-C(4)-H(4A)	111.5
C(5)-C(4)-H(4A)	111.5
C(3)-C(4)-H(4A)	111.5

C(6)-C(5)-C(4)	113.2(3)
C(6)-C(5)-H(5A)	123.4
C(4)-C(5)-H(5A)	123.4
C(5)-C(6)-C(1)	112.4(3)
C(5)-C(6)-H(6A)	123.8
C(1)-C(6)-H(6A)	123.8
O(4)-C(7)-O(5)	125.5(2)
O(4)-C(7)-N(1)	122.7(2)
O(5)-C(7)-N(1)	111.5(2)
O(5)-C(8)-C(9)	111.2(2)
O(5)-C(8)-H(8A)	109.4
C(9)-C(8)-H(8A)	109.4
O(5)-C(8)-H(8B)	109.4
C(9)-C(8)-H(8B)	109.4
H(8A)-C(8)-H(8B)	108.0
C(8)-C(9)-Cl(3)	112.45(19)
C(8)-C(9)-Cl(1)	106.2(2)
Cl(3)-C(9)-Cl(1)	109.39(16)
C(8)-C(9)-Cl(2)	111.5(2)
Cl(3)-C(9)-Cl(2)	107.92(16)
Cl(1)-C(9)-Cl(2)	109.40(15)
O(2)-C(10)-O(3)	104.9(2)
O(2)-C(10)-C(12)	108.6(3)
O(3)-C(10)-C(12)	108.6(2)
O(2)-C(10)-C(11)	110.6(3)
O(3)-C(10)-C(11)	110.1(3)
C(12)-C(10)-C(11)	113.7(3)
C(10)-C(11)-H(11A)	109.5
C(10)-C(11)-H(11B)	109.5
H(11A)-C(11)-H(11B)	109.5
C(10)-C(11)-H(11C)	109.5
H(11A)-C(11)-H(11C)	109.5
H(11B)-C(11)-H(11C)	109.5
C(10)-C(12)-H(12A)	109.5
C(10)-C(12)-H(12B)	109.5
H(12A)-C(12)-H(12B)	109.5

C(10)-C(12)-H(12C)	109.5
H(12A)-C(12)-H(12C)	109.5
H(12B)-C(12)-H(12C)	109.5

Symmetry transformations used to generate equivalent atoms:

Table 11- Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **166**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
Br(1)	16(1)	24(1)	26(1)	0(1)	6(1)	2(1)
Cl(1)	52(1)	44(1)	20(1)	-7(1)	-3(1)	-4(1)
Cl(2)	30(1)	41(1)	29(1)	6(1)	9(1)	-5(1)
Cl(3)	28(1)	37(1)	31(1)	0(1)	6(1)	7(1)
O(1)	13(1)	35(1)	16(1)	4(1)	3(1)	-3(1)
O(2)	22(1)	40(1)	20(1)	-2(1)	9(1)	1(1)
O(3)	22(1)	35(1)	16(1)	-4(1)	4(1)	3(1)
O(4)	19(1)	52(2)	28(1)	-3(1)	4(1)	-11(1)
O(5)	20(1)	28(1)	16(1)	2(1)	3(1)	-3(1)
N(1)	12(1)	33(2)	18(1)	3(1)	3(1)	-4(1)
C(1)	15(1)	20(1)	13(1)	0(1)	4(1)	-1(2)
C(2)	18(1)	20(1)	18(1)	0(1)	5(1)	2(1)
C(3)	21(1)	26(2)	18(1)	2(1)	7(1)	4(1)
C(4)	18(1)	34(1)	18(1)	2(2)	6(1)	-1(2)
C(5)	28(2)	26(2)	16(1)	-1(1)	10(1)	-5(1)
C(6)	27(2)	19(1)	14(1)	1(1)	7(1)	-1(1)
C(7)	21(1)	22(1)	20(1)	2(1)	5(1)	0(1)
C(8)	32(2)	25(1)	18(1)	-3(1)	2(1)	-3(1)
C(9)	24(1)	29(2)	19(1)	-4(1)	3(1)	-2(1)
C(10)	25(1)	38(2)	20(1)	-5(1)	8(1)	2(1)
C(11)	44(2)	48(2)	42(2)	-19(2)	17(2)	2(2)
C(12)	41(2)	64(3)	23(2)	5(2)	9(1)	3(2)

Table 12- Hydrogen coordinates ($x10^4$) and isotropic displacement parameters ($Å^2 x\ 10^3$) for **166**.

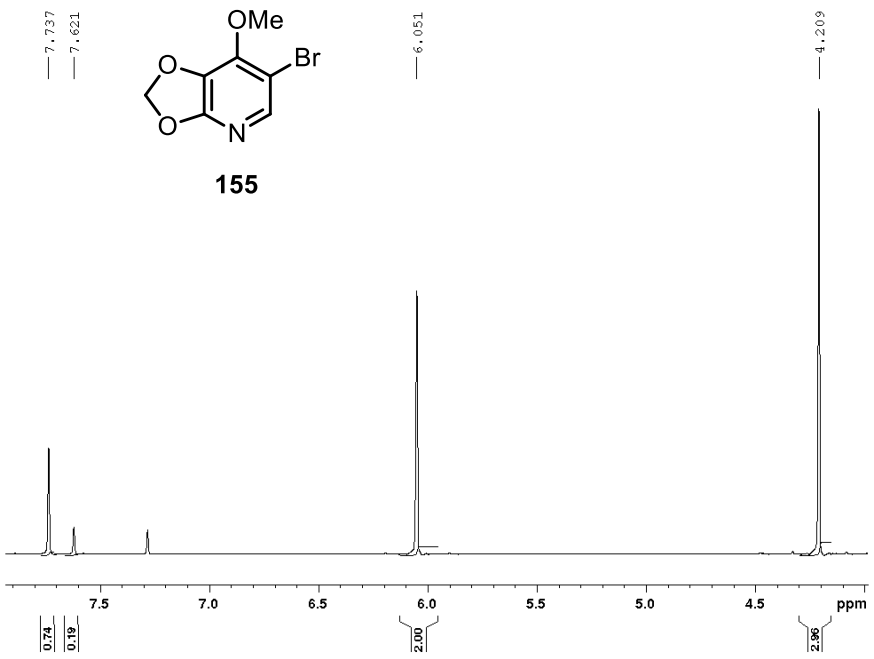
	x	y	z	U(eq)
H(2A)	5171	3079	3617	22
H(3A)	7836	3133	3732	25
H(4A)	9220	6102	4259	28
H(5A)	7819	8972	3638	27
H(6A)	5020	8933	3472	23
H(8A)	7858	7651	7926	31
H(8B)	9495	7443	7574	31
H(11A)	5103	1799	1136	65
H(11B)	6996	1883	1285	65
H(11C)	6223	1378	2198	65
H(12A)	4843	5180	507	63
H(12B)	5764	6864	1184	63
H(12C)	6728	5399	651	63

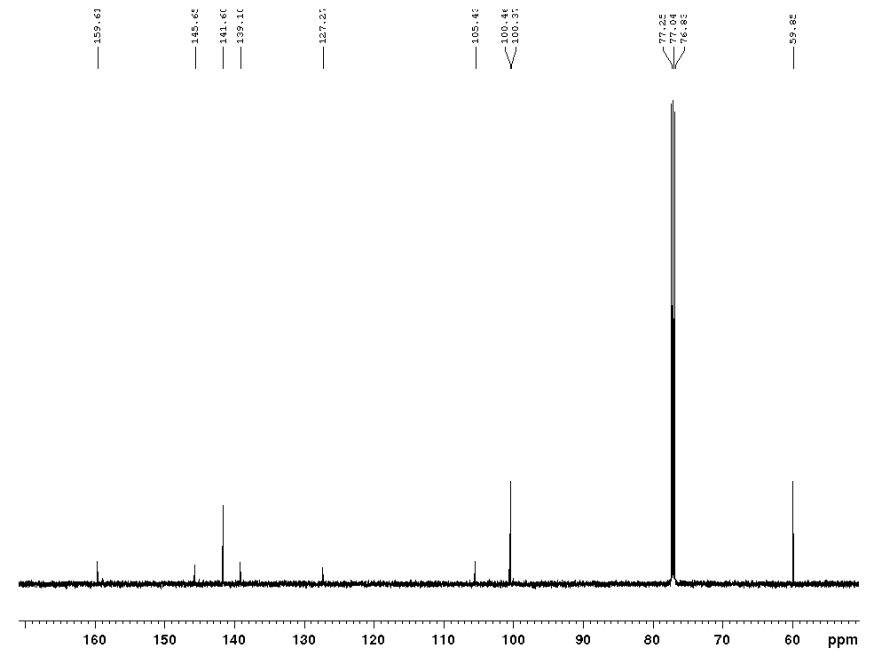
Table 13- Torsion angles [°] for **166.**

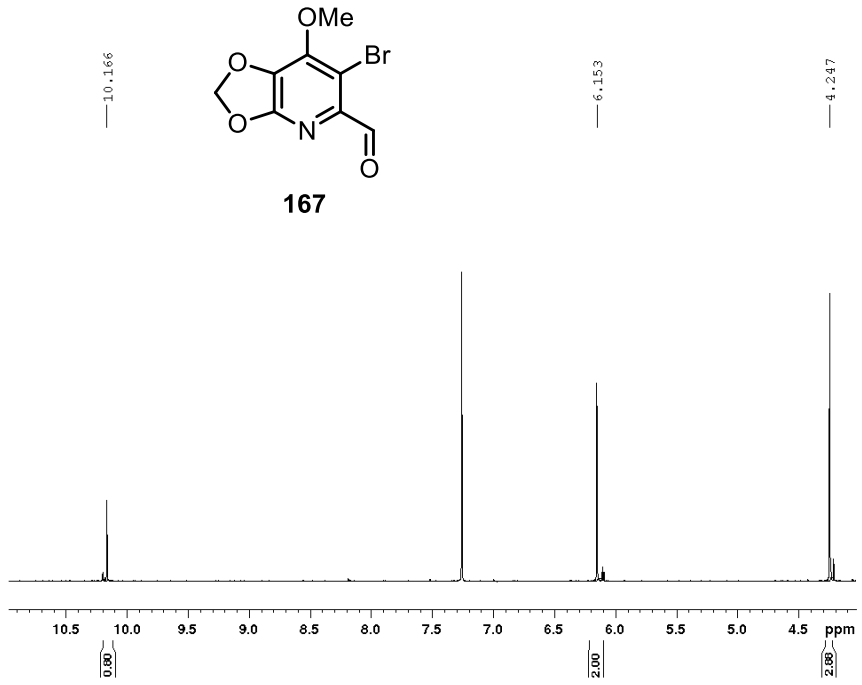
C(1)-O(1)-N(1)-C(7)	146.6(3)
C(1)-O(1)-N(1)-C(4)	1.5(3)
N(1)-O(1)-C(1)-C(6)	-57.1(3)
N(1)-O(1)-C(1)-C(2)	64.2(3)
N(1)-O(1)-C(1)-Br(1)	-178.84(16)
C(10)-O(3)-C(2)-C(1)	132.5(2)
C(10)-O(3)-C(2)-C(3)	17.4(3)
O(1)-C(1)-C(2)-O(3)	-178.5(2)
C(6)-C(1)-C(2)-O(3)	-58.7(3)
Br(1)-C(1)-C(2)-O(3)	69.9(3)
O(1)-C(1)-C(2)-C(3)	-64.5(3)
C(6)-C(1)-C(2)-C(3)	55.3(3)
Br(1)-C(1)-C(2)-C(3)	-176.07(17)
C(10)-O(2)-C(3)-C(4)	-138.1(2)

C(10)-O(2)-C(3)-C(2)	-21.5(3)
O(3)-C(2)-C(3)-O(2)	2.4(3)
C(1)-C(2)-C(3)-O(2)	-115.5(2)
O(3)-C(2)-C(3)-C(4)	119.2(2)
C(1)-C(2)-C(3)-C(4)	1.4(3)
C(7)-N(1)-C(4)-C(5)	-90.4(3)
O(1)-N(1)-C(4)-C(5)	53.1(3)
C(7)-N(1)-C(4)-C(3)	152.6(2)
O(1)-N(1)-C(4)-C(3)	-63.9(3)
O(2)-C(3)-C(4)-N(1)	173.1(2)
C(2)-C(3)-C(4)-N(1)	59.4(3)
O(2)-C(3)-C(4)-C(5)	57.6(3)
C(2)-C(3)-C(4)-C(5)	-56.1(3)
N(1)-C(4)-C(5)-C(6)	-54.5(3)
C(3)-C(4)-C(5)-C(6)	57.9(3)
C(4)-C(5)-C(6)-C(1)	0.1(3)
O(1)-C(1)-C(6)-C(5)	58.0(3)
C(2)-C(1)-C(6)-C(5)	-59.1(3)
Br(1)-C(1)-C(6)-C(5)	173.49(19)
C(8)-O(5)-C(7)-O(4)	7.1(5)
C(8)-O(5)-C(7)-N(1)	-178.5(2)
O(1)-N(1)-C(7)-O(4)	-165.7(3)
C(4)-N(1)-C(7)-O(4)	-23.3(5)
O(1)-N(1)-C(7)-O(5)	19.7(4)
C(4)-N(1)-C(7)-O(5)	162.1(3)
C(7)-O(5)-C(8)-C(9)	-107.5(3)
O(5)-C(8)-C(9)-Cl(3)	64.7(3)
O(5)-C(8)-C(9)-Cl(1)	-175.71(18)
O(5)-C(8)-C(9)-Cl(2)	-56.6(3)
C(3)-O(2)-C(10)-O(3)	32.7(3)
C(3)-O(2)-C(10)-C(12)	148.6(2)
C(3)-O(2)-C(10)-C(11)	-86.0(3)
C(2)-O(3)-C(10)-O(2)	-31.1(3)
C(2)-O(3)-C(10)-C(12)	-147.0(3)
C(2)-O(3)-C(10)-C(11)	87.9(3)

7. Selected Spectra



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O
N
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5.0
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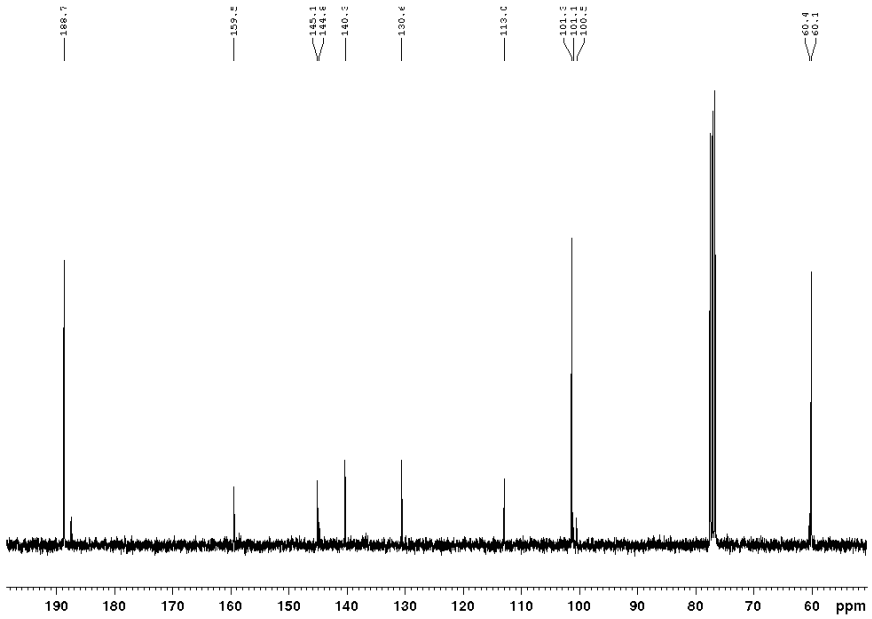
2.00

167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180
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160
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140
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120
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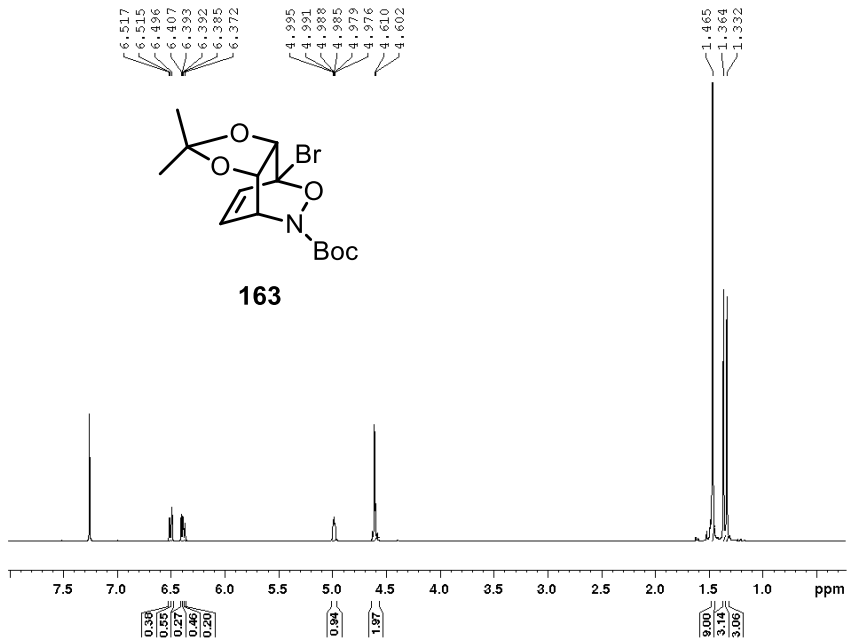
80
70
60
ppm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6.515
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4.979
4.976
4.610
4.602
1.465
1.364
1.332
O
O
Br
O
N
Boc
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
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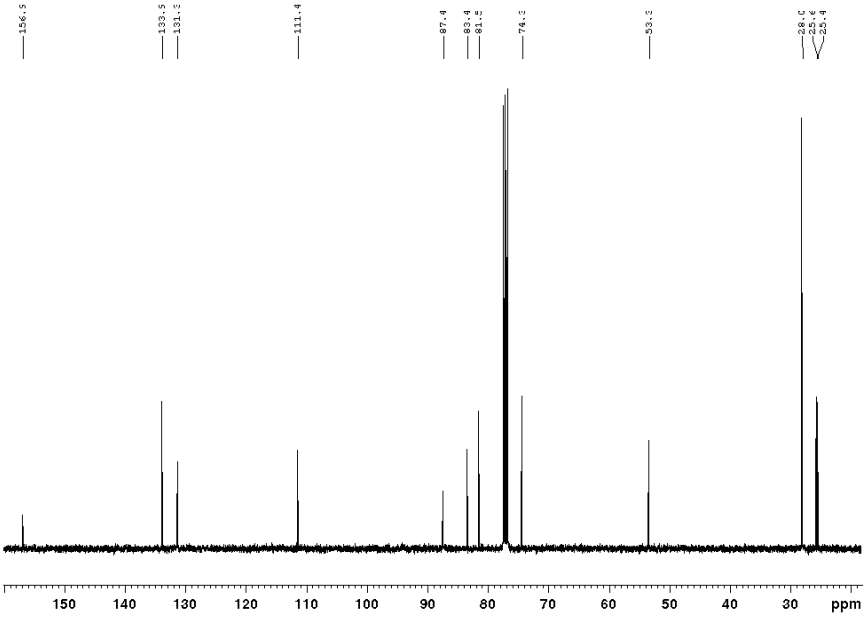
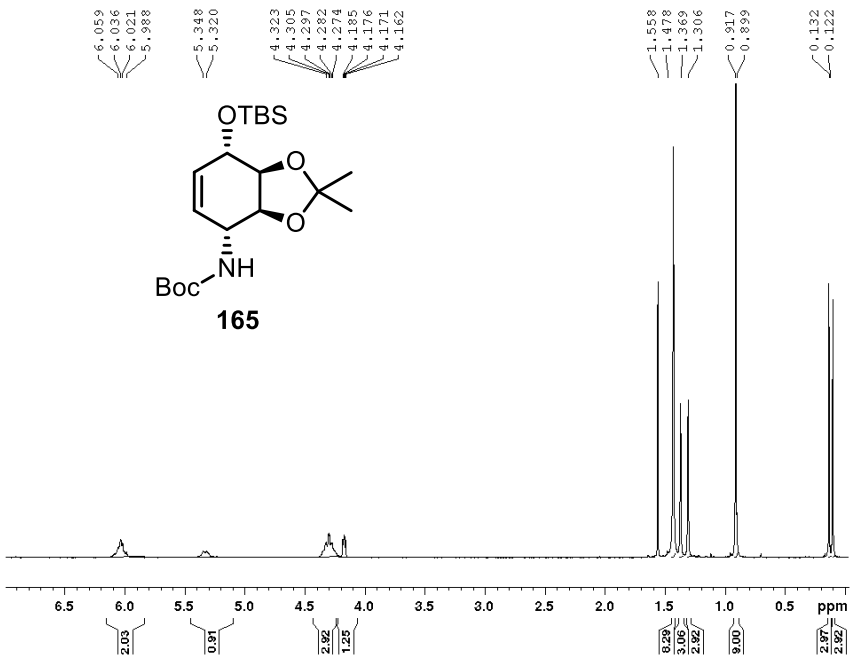
0.55
0.27
0.46
0.20
0.94
1.97
9.00
3.14
3.06

163


156.5
133.5
131.3
111.4
87.4
83.4
81.8
74.3
53.3
28.0
25.6
25.4
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm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5.348
5.320
4.323
4.305
4.297
4.282
4.274
4.185
4.176
4.171
4.162
1.558
1.478
1.369
1.306
0.917
0.899
0.132
0.122
OTBS
O
O
NH
Boc
165
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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ppm
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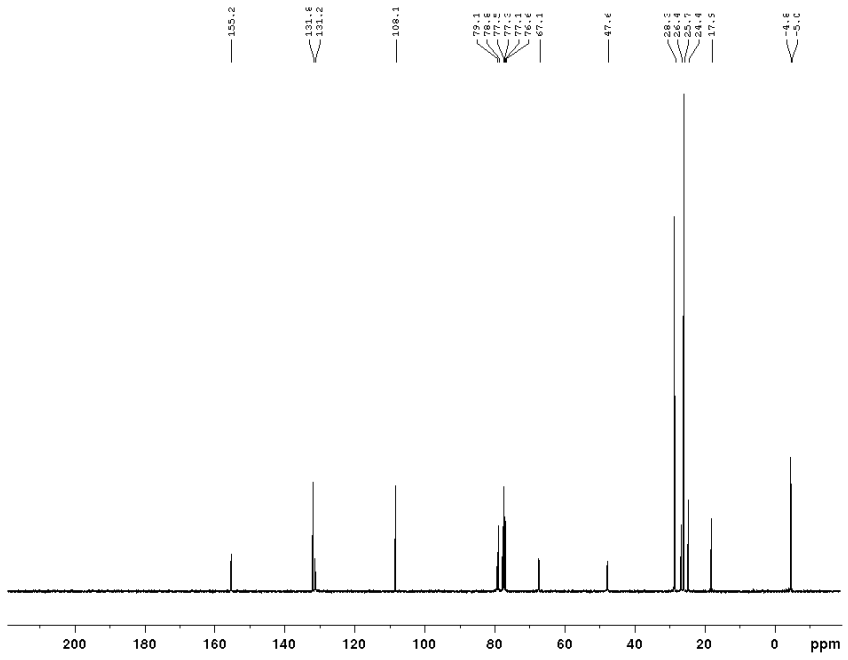
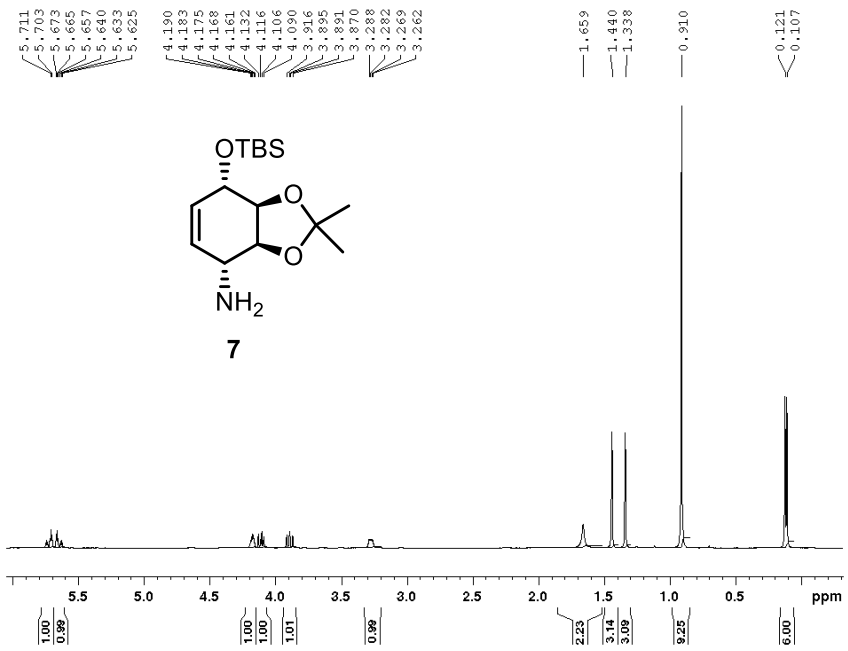
0.91
2.92
1.25
8.29
3.06
2.92
9.00
2.97
2.92


155.2
131.6
131.2
108.1
79.1
78.8
77.5
77.3
77.1
76.8
67.1
47.6
28.3
26.4
25.3
24.4
17.5
-4.6
-5.0
200
180
160
140
120
100
80
60
40
20
0
ppm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5.703
5.673
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5.657
5.640
5.633
5.625
4.190
4.183
4.175
4.168
4.161
4.132
4.116
4.106
4.090
3.916
3.895
3.891
3.870
3.288
3.282
3.269
3.262
1.659
1.440
1.338
0.910
0.121
0.107
OTBS
O
O
NH2
7
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
1.00
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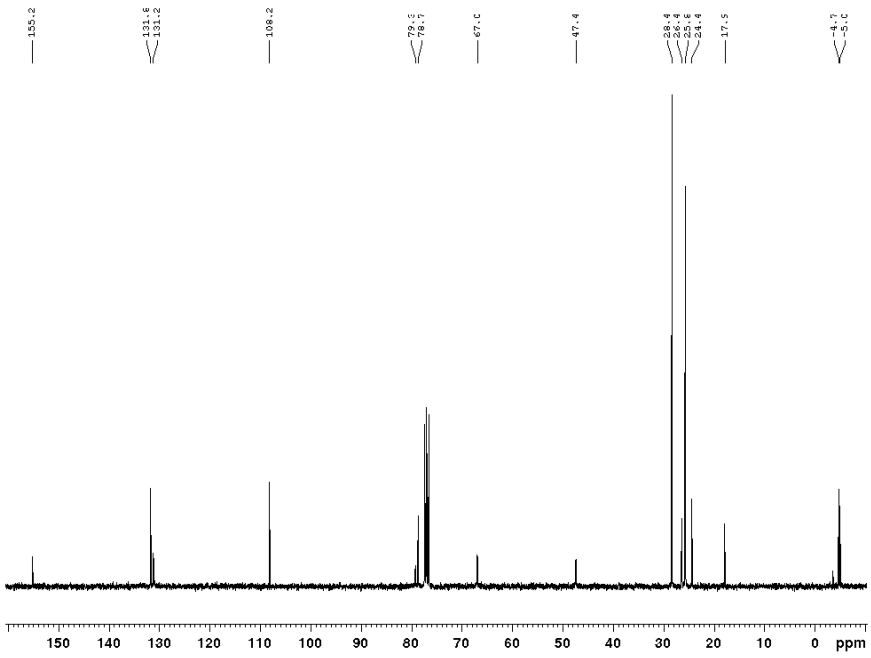
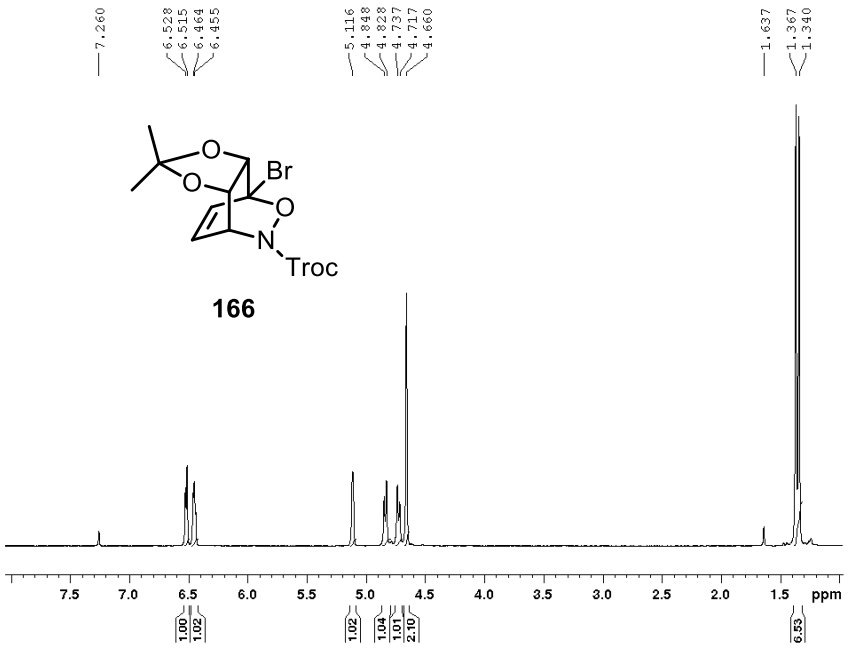
1.00
1.00
1.01
0.99
2.23
3.14
3.09
9.25
6.00


155.2
131.6
131.2
108.2
79.5
78.7
67.0
47.4
28.4
26.4
25.6
24.4
17.5
-4.7
-5.0
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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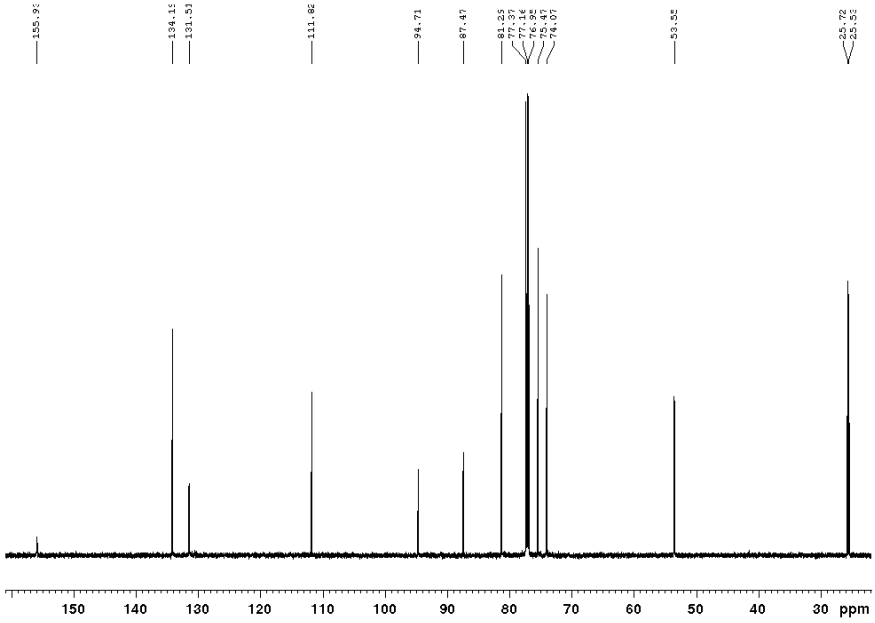
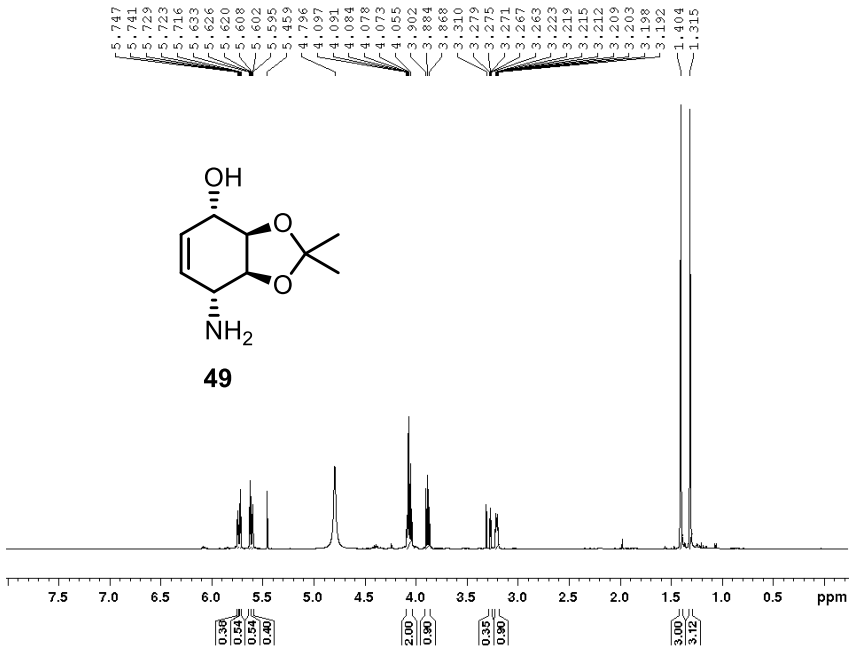
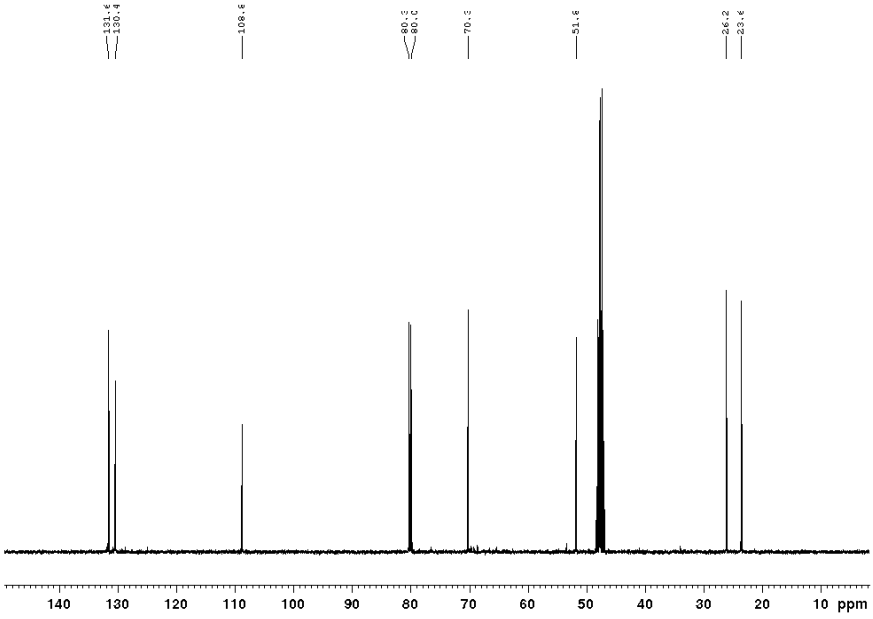
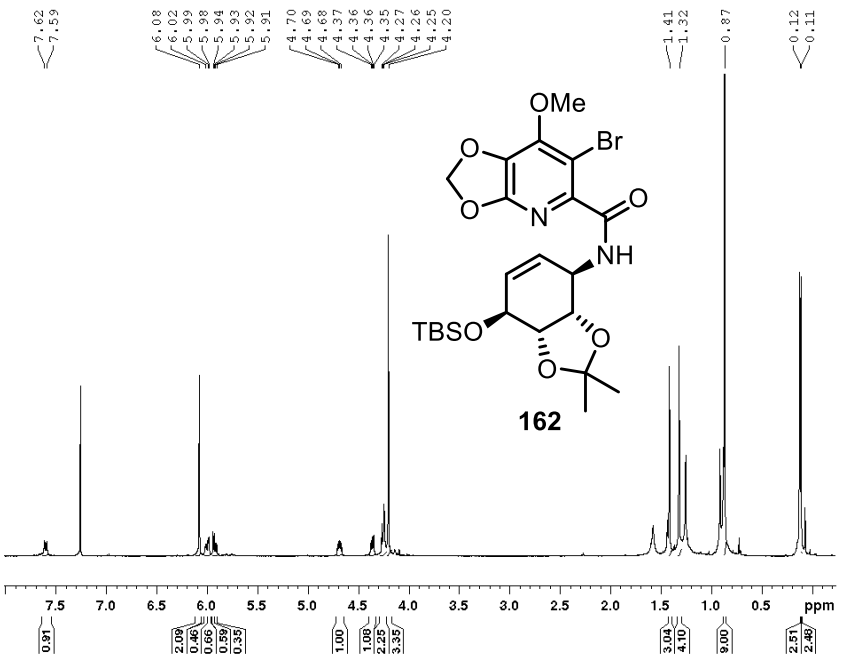
6.528
6.515
6.464
6.455
5.116
4.848
4.828
4.737
4.717
4.660
1.637
1.367
1.340
O
O
Br
O
N
Troc
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm
1.00
1.02
1.02
1.04
1.01
2.10
6.53

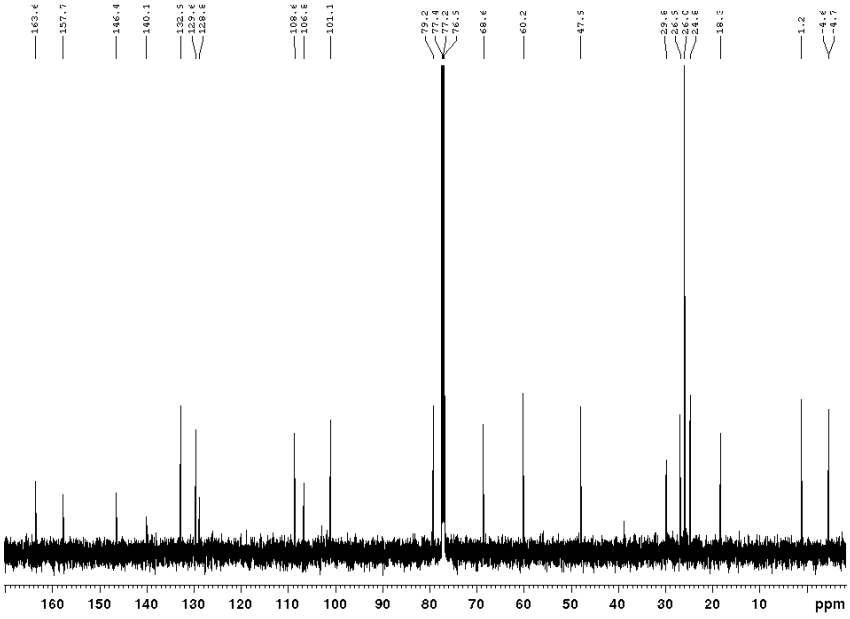
166


155.9
134.1
131.5
111.9
94.71
87.47
81.25
77.37
77.14
76.92
75.47
74.07
53.58
25.72
25.52
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm


5.747
5.741
5.729
5.723
5.716
5.633
5.626
5.620
5.608
5.602
5.535
5.459
4.796
4.097
4.091
4.084
4.078
4.073
4.055
3.902
3.884
3.868
3.310
3.279
3.275
3.271
3.267
3.263
3.223
3.219
3.215
3.212
3.209
3.203
3.198
3.192
1.404
1.315
OH
O
O
NH2
49
7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 ppm
0.36 0.54 0.54 0.40 2.00 0.90 0.35 0.90 3.00 3.12


131.6
130.4
108.6
80.3
80.2
70.3
51.8
26.2
23.4
140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm


7.62
7.59
6.08
6.02
5.99
5.98
5.94
5.93
5.92
5.91
4.70
4.69
4.68
4.37
4.36
4.36
4.35
4.27
4.26
4.25
4.20
1.41
1.32
0.87
0.12
0.11
OMe
Br
O
O
N
O
NH
TBSO
O
O
162
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
ppm
0.91
2.09
0.46
0.66
0.59
0.35
1.00
1.08
2.25
3.35
3.04
4.10
9.00
2.51
2.48


163.6
157.7
146.4
140.1
132.5
129.6
128.6
108.6
106.8
101.1
79.2
77.4
77.2
76.5
68.6
60.2
47.5
29.6
26.5
26.0
24.8
18.3
1.2
-4.6
-4.7
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

8. References

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9. Vita

Zemane W'Giorgis was born in Winnipeg, Manitoba on July 18, 1990. She attended high school at Kelvin High School, before moving onto university studies in University of Winnipeg, Winnipeg, Manitoba. During undergraduate studies she studied under supervision of Dr. Tabitha E. Wood and Dr. Adam McCubbin. In 2013 she moved to St Catharines, Ontario to pursue graduate studies under tutelage of Professor Tomas Hudlický at Brock University. She is presently working towards completion of her MSc degree in organic chemistry. Her research interests include the synthesis of heterocycles and total synthesis of natural products.